A CROSS SECTIONAL STUDY ON THE STATUS OF INFLAMMATORY MARKERS IN POLYCYSTIC OVARY SYNDROME (PCOS) PATIENTS IN INDIAN POPULATION

DISSERTATION

SUBMITTED FOR

M.D. IN PHARMACOLOGY

THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY

DEPARTMENT OF PHARMACOLOGY

PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH

PEELAMEDU, COIMBATORE- 641 004

TAMILNADU, INDIA

APRIL 2015

PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH

COIMBATORE

CERTIFICATE

This is to certify that this dissertation entitled **"A CROSS SECTIONAL STUDY ON THE STATUS OF INFLAMMATORY MARKERS IN POLYCYSTIC OVARY SYNDROME (PCOS) PATIENTS IN INDIAN POPULATION"**, is a work done by **Dr. R. SENTHURSELVI,** Postgraduate under the guidance of **Dr. K. BHUVANESWARI, M.D.,** Professor and Head, Department of Pharmacology, PSG IMS&R.

Dr. K.Bhuvaneswari M.D
Guide, Professor and Head,
Department of Pharmacology,
PSG IMS&R.

Dr.S.Ramalingam M.D
Principal,
PSG IMS&R.

DECLARATION

I solemnly declare that the dissertation titled **"A CROSS SECTIONAL STUDY ON THE STATUS OF INFLAMMATORY MARKERS IN POLYCYSTIC OVARY SYNDROME (PCOS) PATIENTS IN INDIAN POPULATION"** was done by me under the guidance and supervision of **Dr. K. BHUVANESWARI, M.D.,**

This dissertation is submitted to the Tamilnadu Dr.MGR Medical University towards the partial fulfillment of the requirement for the award of M.D Degree in Pharmacology.

Place: **Dr. R. SENTHURSELVI**

Date:

PSG Institute of Medical Sciences & Research
Institutional Human Ethics Committee

Recognized by The Strategic Initiative for Developing Capacity in Ethical Review (SIDCER)
POST BOX NO. 1674, PEELAMEDU, COIMBATORE 641 004, TAMIL NADU, INDIA
Phone : 91 422 - 2598822, 2570170, Fax : 91 422 - 2594400, Email : ihec@psgimsr.ac.in

May 06, 2014

To
Dr R Senthurselvi
Postgraduate
Department of Pharmacology
PSG IM & R
Coimbatore

Ref.: Proposal titled: *"A Cross-sectional study on the status of inflammatory markers in Polycystic Ovary Syndrome (PCOS) patients in Indian population"*

Sub.: Ethics Committee Approval for the study

The Institutional Human Ethics Committee, PSG IMS & R, Coimbatore -4, has reviewed your proposal on 25th April, 2014 in its full board review meeting held at College Council Room, PSG IMS&R, between 2.00 pm and 3.45 pm, and discussed your application to conduct the study entitled:

"A Cross-sectional study on the status of inflammatory markers in Polycystic Ovary Syndrome (PCOS) patients in Indian population"

The following documents were received for review:

1. Duly filled application form
2. Proposal (Ver 1.1)
3. Informed Consent forms
4. Data collection tool
5. Budget
6. CV

The members who attended the meeting at which your study proposal was discussed are as follows:

Sl. No.	Name of the Member of IHEC	Qualification	Area of Expertise	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
1	Dr. S. Bhuvaneshwari (Member-Secretary, IHEC)	MD	Clinical Pharmacology	Female	Yes	Yes
2	Mrs. Geetha S Kannan	+ 2	Lay person	Female	No	Yes
3	Mr Gowpathy Velappan	BA., BL	Legal Advisor	Male	No	Yes
4	Mrs G Malarvizhi	M Sc	Nursing	Female	Yes	Yes
5	Mr. R. Nandakumar (Vice-Chairperson, IHEC)	BA., BL	Legal Expert	Male	No	Yes
6	Dr. G. Rajendran	DM	Clinician (Cardiology)	Male	Yes	Yes
7	Dr. V. Ramamurthy	Ph D	Biotechnology	Male	Yes	Yes

PSG Institute of Medical Sciences & Research
Institutional Human Ethics Committee

Recognized by The Strategic Initiative for Developing Capacity in Ethical Review (SIDCER)
POST BOX NO. 1674, PEELAMEDU, COIMBATORE 641 004, TAMIL NADU, INDIA
Phone : 91 422 - 2598822, 2570170, Fax : 91 422 - 2594400, Email : ihec@psgimsr.ac.in

8	Dr. M. Ramanathan	M Pharm, Ph D	Non-Medical (Pharmacy)	Male	Yes	Yes
9	Dr. P. Sathyan (Chairperson, IHEC)	DO, DNB	Clinician (Opthalmology)	Male	No	Yes
10	Dr. Seetha Panicker	MD	Clinician (Obstetrics & Gynaecology)	Female	Yes	Yes
11	Dr. S. Shanthakumari	MD	Pathology, Ethicist	Female	Yes	No
12	Dr. Y.S. Sivan	Ph D	Social Scientist (Sociology)	Male	Yes	Yes
13	Dr. Sudha Ramalingam (Alternate Member-Secretary, IHEC)	MD	Public Health, Epidemiology, Genetics, Ethicist	Female	Yes	Yes
14	Mrs. K. Uma Maheswari	M Sc, M Phil, B Ed	Botany	Female	No	Yes
15	Dr. D. Vijaya	M Sc, Ph D	Basic Medical Sciences (Biochemistry)	Female	Yes	Yes

After due consideration, the committee has decided to approve the above proposal.

The approval is valid for one year.

We hereby confirm that neither you nor any of your study team members have participated in the voting/ decision making procedure of the committee. The members of the committee who have participated in the voting/ decision making procedure of the committee do not have any conflict of interest in the referenced study.

This Ethics Committee is organized and operates according to Good Clinical Practice and Schedule Y requirements.

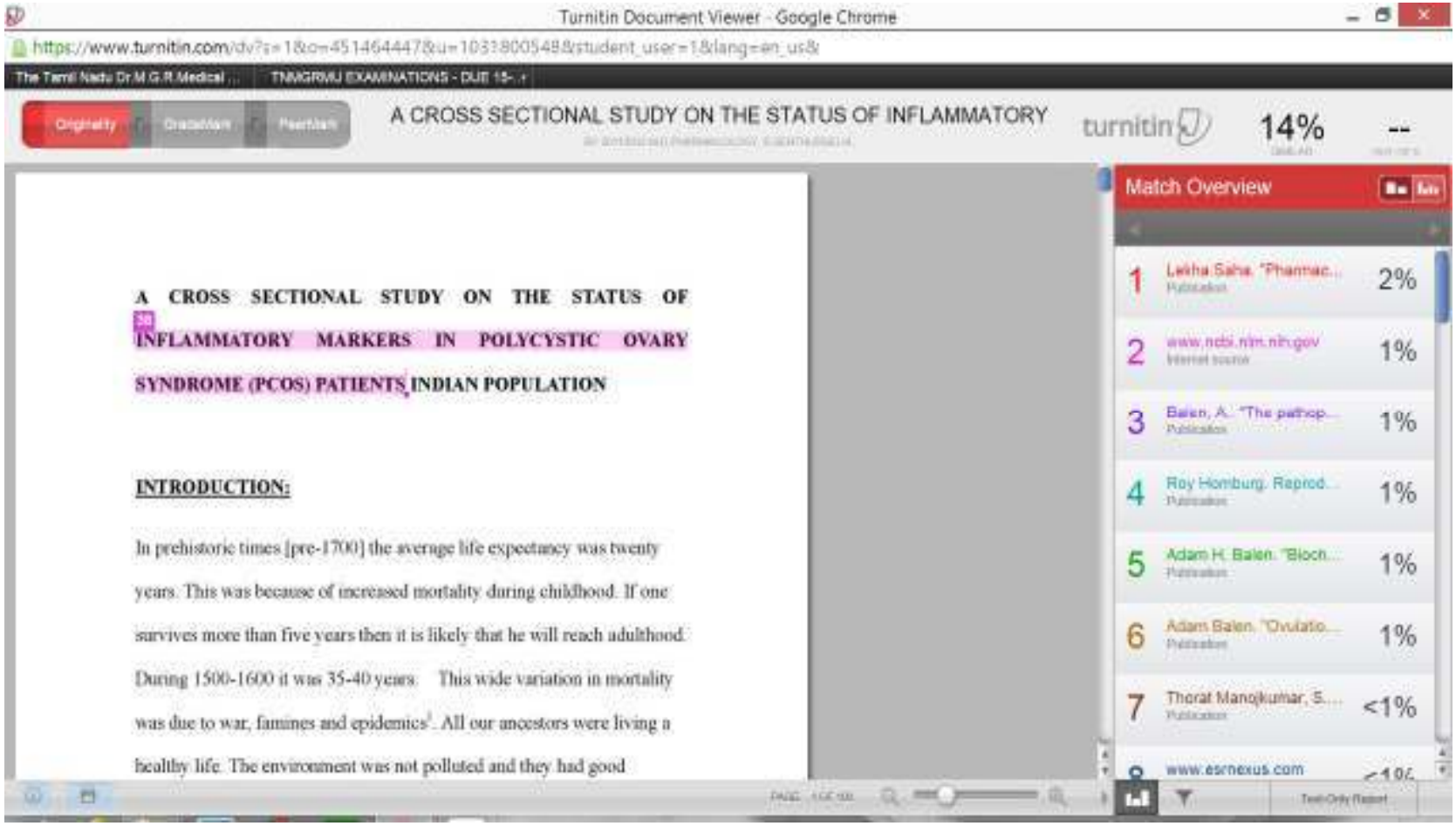
Non-adherence to the Standard Operating Procedures (SOP) of the Institutional Human Ethics Committee (IHEC) and national and international ethical guidelines shall result in withdrawal of approval (suspension or termination of the study). SOP will be revised from time to time and revisions are applicable prospectively to ongoing studies approved prior to such revisions.

PIs are required to send progress reports (in the form of an extended abstract with publications if any) to the IHEC every six months (and a month before expiry of approval date, if renewal of approval is being sought).

Request for renewal must be made at least a month ahead of the expiry of validity along with a copy of the progress report.

SECRETARY PSG IMSAR COIMBATORE-641004 IHEC COMMITTEE

Dr S Bhuvaneshwari
Member - Secretary
Institutional Human Ethics Committee


Turnitin Document Viewer - Google Chrome
https://www.turnitin.com/dv?s=1&o=451464447&u=1031800548&student_user=1&lang=en_us&
A CROSS SECTIONAL STUDY ON THE STATUS OF INFLAMMATORY
turnitin
14%
--
A CROSS SECTIONAL STUDY ON THE STATUS OF INFLAMMATORY MARKERS IN POLYCYSTIC OVARY SYNDROME (PCOS) PATIENTS INDIAN POPULATION
INTRODUCTION:
In prehistoric times [pre-1700] the average life expectancy was twenty years. This was because of increased mortality during childhood. If one survives more than five years then it is likely that he will reach adulthood. During 1500-1600 it was 35-40 years. This wide variation in mortality was due to war, famines and epidemics[1]. All our ancestors were living a healthy life. The environment was not polluted and they had good
Match Overview
1 Lekha Saha, "Pharmac... Publication 2%
2 www.ncbi.nlm.nih.gov Internet source 1%
3 Balen, A. "The pathop... Publication 1%
4 Roy Homburg, Reprod... Publication 1%
5 Adam H. Balen, "Bloch... Publication 1%
6 Adam Balen, "Ovulatio... Publication 1%
7 Thorat Manojkumar, S.... Publication <1%
8 www.escnexus.com <1%

turnitin

Digital Receipt

Page No.

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	20112912.m.d,pharmacology, R.Sen…
Assignment title:	TNMGRMU EXAMINATIONS
Submission title:	A CROSS SECTIONAL STUDY ON…
File name:	dissertation_final-senthur.doc
File size:	480.5K
Page count:	100
Word count:	13,741
Charader count:	77,827
Submission date:	26-Sep-2014 08:56AM
Submission ID:	451464447

A CROSS SECTIONAL STUDY ON THE STATUS OF INFLAMMATORY MARKERS IN POLYCYSTIC OVARY SYNDROME (PCOS) PATIENTS INRURAN POPULATION

INTRODUCTION

CONTENTS

A CROSS SECTIONAL STUDY ON THE STATUS OF INFLAMMATORY MARKERS IN POLYCYSTIC OVARY SYNDROME (PCOS) PATIENTS IN INDIAN POPULATION

ABSTRACT:

AIMS AND OBJECTIVES: To assess the status of inflammation in patients with Polycystic Ovary Syndrome (PCOS) and to assess the influence of drugs prescribed for PCOS on inflammation.

MATERIALS AND METHODS: Patients were divided into three groups as healthy volunteers, newly diagnosed PCOS patients, already on treatment patients for PCOS. hs-CRP, IL-6 and IL-18 were assessed.

RESULTS: There was an increase in the hs-CRP, IL-6 and IL-18 compared to healthy volunteers in both newly diagnosed and already diagnosed groups. But there was no statistical significance (hs-CRP=0.975, IL-6=0.746, IL-18 = 0.148) between them. The mean values has decreased in the already on treatment group as duration of treatment increases. There was no statistical significance between them.

CONCLUSIONS: There was an inversely proportional relationship between the mean values of inflammatory markers and duration of treatment.

INTRODUCTION:

In prehistoric times [pre-1700] the average life expectancy was twenty years. This was because of increased mortality during childhood. If one survives more than five years then it is likely that he will reach adulthood. During 1500-1600 it was 35-40 years. This wide variation in mortality was due to war, famines and epidemics[1]. All our ancestors were living a healthy life. The environment was not polluted and they had good physical activity. Their food was free of pesticides.

In today's modern world we follow all hygienic practices right from our home. By following all these practices we are able to bring down the mortality rate and increase the life expectancy. On the other hand in this modern world physical activity is reduced and all edible products we consume are treated with pesticides. We also lead a stressful life. All these leads to increased morbidity, which is what we pay to this modern world for increasing our life expectancy. Both men and women are equally affected and some disease are specific for either men or women. One among them is **Polycystic Ovarian Syndrome (PCOS)** which is seen exclusively in women of reproductive age group.

World Health Organization reports that Polycystic Ovarian Syndrome approximately affects 5-10% of the reproductive female population worldwide. A study by Ridhi R et al. shows it affects the Indian female adolescents approximately by 9.13%[2].

Women with PCOS have chronic inflammation. This in turn leads to increased incidence of non-communicable diseases like cardiovascular disease and diabetes[3]. **Chronic inflammation** in PCOS patients can be identified using serum markers such as C - reactive protein [CRP], Interleukin-6[IL-6] and Interleukin-18 [IL-18].

We did a thorough search of literature in subscribed journals of our institute (print and online), PubMed, Ovid, Google, etc., to look for the status of inflammatory markers in Polycystic Ovary Syndrome patients. Almost all the publications are from the western world (Europe). There was only one study from India on the women with Polycystic Ovary Syndrome showing the status of Inflammation mainly the atherosclerotic markers[3]. All PCOS patients undergo routine investigations like Ultrasound abdomen and blood investigations.

This study was basically carried out to see if there is any association between inflammation and PCOS. If the serum inflammatory markers are higher, then like western population, there is an association between

inflammation and PCOS in Indian Population. We would also like to see drugs used in PCOS has any influence over the inflammation in PCOS.

AIMS & OBJECTIVES:

1. To assess the status of inflammation in patients with Polycystic Ovary Syndrome (PCOS)
2. To assess the influence of drugs prescribed for PCOS on inflammation

REVIEW OF LITERATURE

PCOS is a disorder with varied presentation which form a wide range of signs and symptoms. The presentation may be mild in some and severe in others with respect to reproductive, endocrine and metabolic functions. The prevalence of polycystic ovaries in general population is 20-33% detected using ultrasound or other pelvic imaging modalities [4].

The first histological description of polycystic ovary and features of the condition was made by Vallisneri in the year 1721. In 1935, it was **Stein Sr.** and **Leventhal**, two American Gynecologists saw presence of ovarian cysts along with anovulation. They described it as Polycysic Ovary Syndrome (PCOS). Initially presence of ovarian cysts with anovulation was the investigative measures of the disease.Anovulation and increased levels of androgens but not the ovarian cysts were made the diagnostic criteria later. The histology of polycystic ovary was of an ovary with prominent theca, fibrotic thickening of the tunica albuginea and multiple cystic follicles[5]. For many years wedge resection was the only treatment for PCOS and histological assessment of the ovaries was therefore routine practise. The histopathological criteria have been defined as the observation of: atretic follicle and/or degenerating

granulosa cell, hypertrophy and leutenisation of the inner theca cell layer, and thickened ovarian tunica[6].

Ultrasound came into the picture during early 1980's by using high-resolution real-time sector scanners[7,8]. Using ultrasound ovarian appearance was described in women classified as having PCOS (by symptoms and serum endocrinology). Orsini et al described ovaries as either being predominantly solid if fewer than four small (<9mm) cystic structures were detected in the ovary or predominantly cystic if multiple cystic structures or atleast one large cyst (>10mm) were present[9]. Adams et al. defined a polycystic ovary as in one plane, atleast 10 follicles (usually between 2 and 8mm in diameter) arranged peripherally around a thick core of ovarian stroma or dispersed all over in an increased amount of stroma according to an abdominal ultrasound[10]. When scattered throughout the stroma the cysts were usually between 2-4mm in diameter[11]. The criteria of Adams and colleagues have been implemented by various subsequent reports which used this criteria to identify polycystic ovaries[3, 12, 13-18]. Abder Gadir et al found that the visualization of the polycystic ovaries supported the diagnosis of the syndrome in women with signs and symptoms, rather than being key in making the diagnosis[16].

After examining tissues of ovary at hysterectomy or wedge resection it was found to have a good correlation between ultrasound diagnosis of polycystic morphology and histopathological criteria for polycystic ovaries [19, 20].

It was recognized that PCOS is a heterogeneous condition and several large series exist that describe populations of patients with the condition. The components that define PCOS should include no less than two of the following- menstrual disturbance (oligo-anovulation), hyperandrogenism and polycystic ovaries. The first largest series of polycystic ovaries women was reported by Balen et al who discovered this spectrum of the disorder in 1741 patients[21]. 38% of the women were overweight. Obesity was related with increased possibility of hirsutism, menstrual cycle abnormality and increased rate of infertility. The frequency of obesity was reliably large in most of the reports and most of them with polycystic ovaries were found to have menstrual cycle abnormalities than thin women with polycystic ovaries[21, 22]. Conway et al proved mean ovarian volume was higher in polycystic ovaries women who are diagnosed through ultrasound than the control group[23]. Franks et al established a higher area of uterus and mean ovarian volume was doubled in the PCOS group[22]. High serum LH (luteinizing hormone)

concentration were linked with infertility or menstrual cycle irregularities in both Frank and Balen study. Balen et al also identified that elevated levels of serum testosterone were related to increased potential of hirsutism, infertility and menstrual abnormalities. Ovarian volume strongly associated with serum levels of LH and testosterone concentration.

The incidence of PCOS are highly related to the assessing population's nature. A cross-sectional study by Knochenhauer et al examined the prevalence of PCOS in a population of American women to be 4%[24]. There is ethnic variation in the prevalence of PCOS. The insulin resistance (IR) and symptoms were more in anovular South Asians than Caucasians with PCOS[25].

In PCOS patients ovary is the primary site of endocrine abnormality particularly hyperandrogenism. It was Rosenfield et al who mentioned that disorder of P450c17α plays a vital role in overproduction of androgen in ovaries[26]. It was later proved by others by injecting one dose of gonadotrophin releasing hormone agonist (GnRHa), naferalin to hyperandrogenic PCOS women in whom secretion of adrenal androgen had been inhibited by giving dexamethasone and their pituitary and ovarian response was found[27]. Franks et al extended this study to

anovulatory and ovulating hyperandrogenemic women and reported a little but substantial rise in androstenedione levels in both groups in reaction to GnRHa and an alike response in 17-hydroxyprogesterone levels, which were considerably higher in the women with anovulation[28]. The elevation of these two hormones was not present when ACTH injection was administerd. These results shows that hyperandrogenemia in both ovulatory and anovulatory women with PCOS is mostly of ovarian origin.

The distinctive morphology of ovaries is pathognomonic of this disease, its chief marker being hyperandrogenemia rising from the theca cells. Development of follicles is concerned with antral follicles detained at a diameter of 2-9mm. Study of follicle densities showed that normal ovaries had 11.4 small preantral follicles/m^3 (4-34), ovulatory polycystic ovaries had a density of 27.4 follicles/m^3 (9-81), and anovulatory polycystic ovaries had a density of 73.0 follicles/m^3 (31-94). This significant difference was also demonstrated for the primary follicles[29].

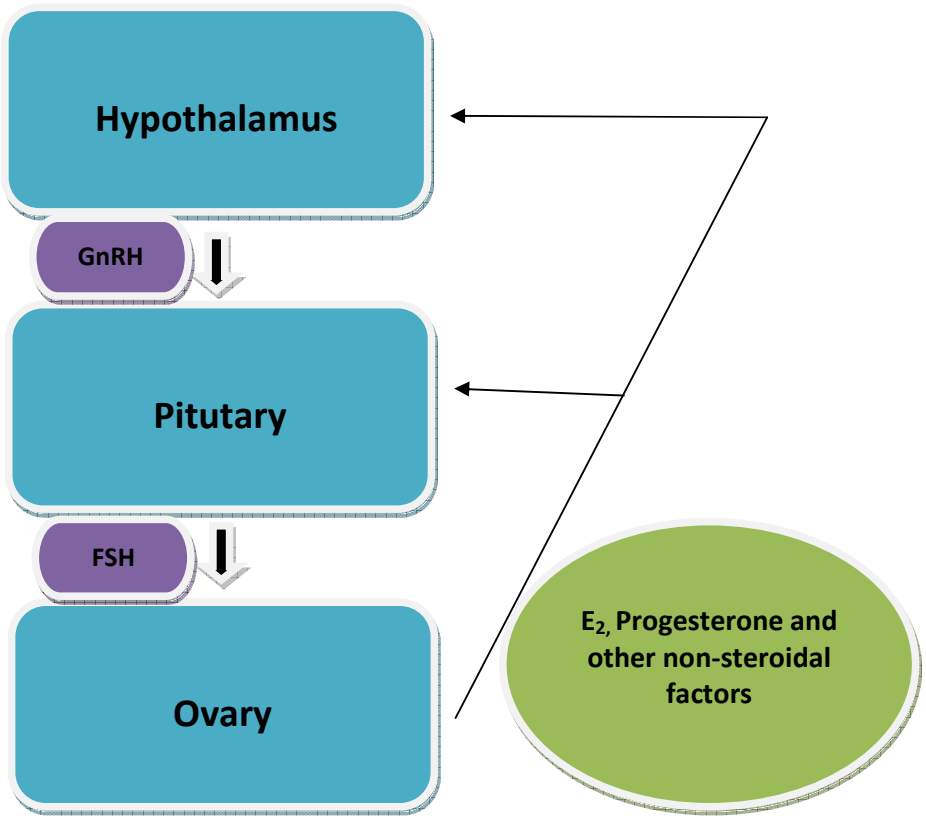
Serum LH concentrations are considerably higher in PCOS women than the controls[30,31]. This is because amplitude and frequency of LH pulses are elevated[32]. LH concentrations are increased which are seen in approximately 40-60% of PCOS women[32,33,34]. Raised serum LH

concentrations has been related todecreased chance of conception and high risk of miscarriage[35]. LH levels are predisposed by the temporal relation to ovulation, which quickly normalizes LH due to the suppressive effect of progesterone.

The pituitary gonadotropin is foremost to the function of reproduction. Its production and secretion of LH and FSH is directly stimulated by hypothalamic GnRH and is also affected by integrated feedback mechanism. FSH affords the early spur for follicular development and also helps alteration of androgens to estrogens in granulosa cells by stimulating the aromatase enzymes. LH plays a part in luteal phase by stimulating secretion of progesterone, also has a central role in the follicular phase, persuading thecal androgen production (substrate for estrogen synthesis) and initiating oocyte maturation at mid-cycle.

A single hypothalamic decapeptide, GnRH stimulates the gonadotroph to release both LH and FSH from the gonadotroph[36]. Pulsatile GnRH stimulation is mandatory to maintain gonadotroph secretion. When GnRH pulsatility is sluggish FSH secretion outweighs, and when rapid LH secretion dominates[37].

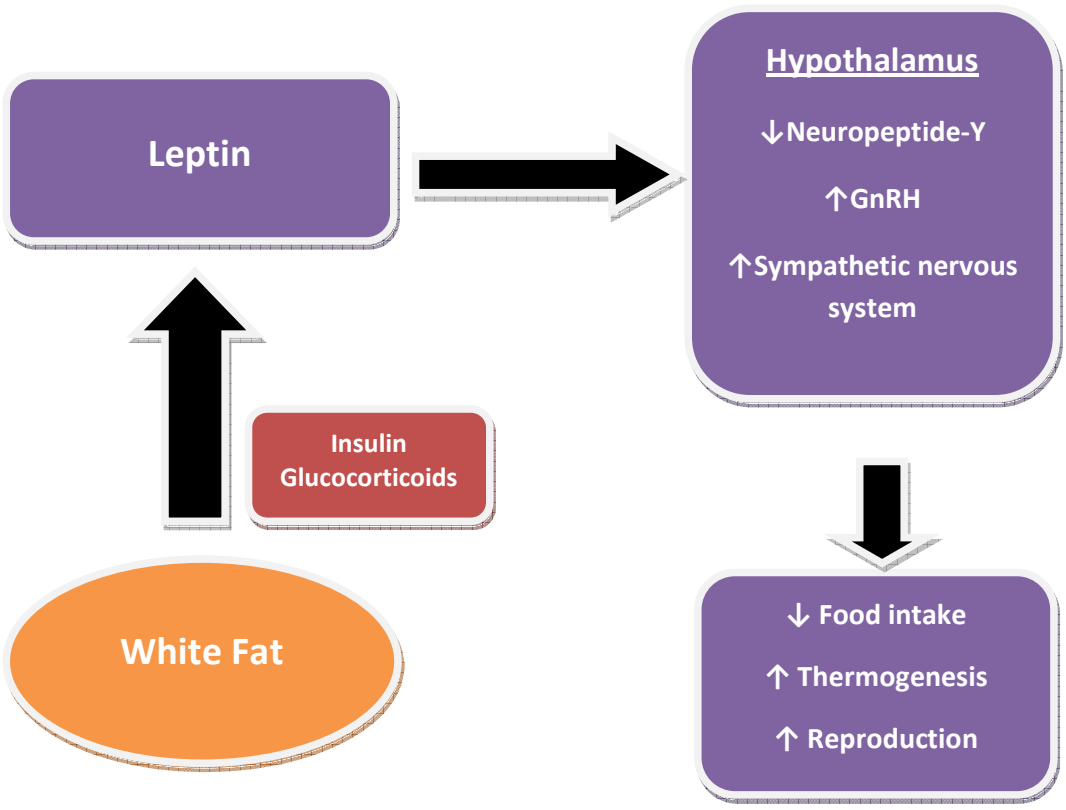
Picture1:Feedback Mechanism of FSH and GnRH on Hypothalamus



There are various causes that impact GnRH activity which comprise β-endorphin, estradiol (E_2), angiotensin II, melatonin, serotonin, neuropeptide-Y, oxytocin, neurotensin, somatostatin, dopamine and substance-P.

The increasing prevalence of obesity in western society should increase our awareness of the possibility of PCOS developing in genetically susceptible individuals. The association between obesity and raised free androgen levels is strong in women with increased central obesity.

Picture2:Functions of Leptin



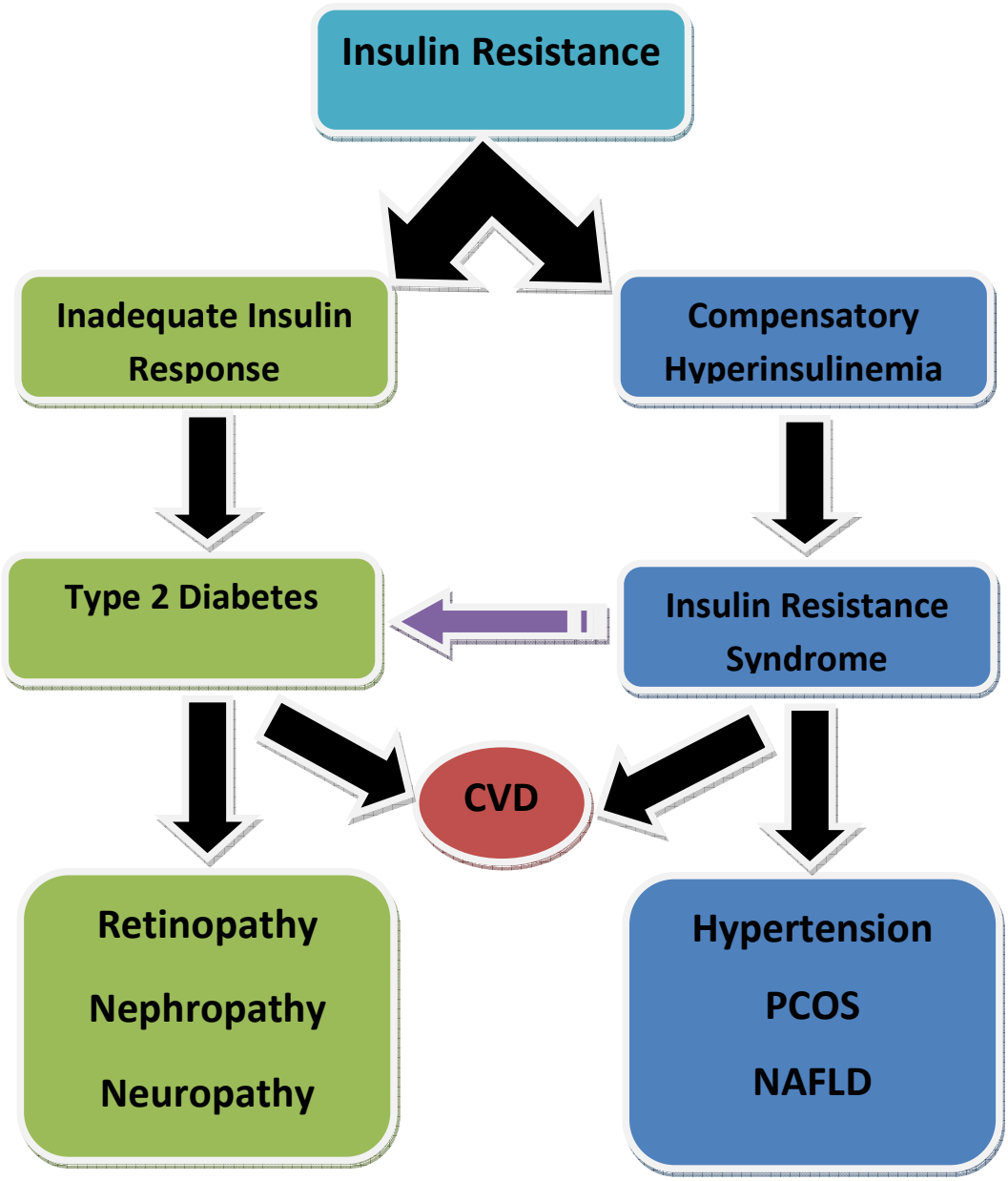
Leptin is a 167 aminoacid peptide that is produced by the fat cells in reaction to insulin and glucocorticoids. Leptin is conveyed by a protein which appears to be the extracellular domain of the leptin receptor itself[38]. Leptin declines food intake and induces thermogenesis, it also seems to block the hypothalamic neuropeptide-Y, an inhibitor of GnRH pulsatility. Leptin appears to aid as the indicator from the body fat to the brain about the availability of fat stores for reproduction. Consequently menstruation will happen if stores of fat are sufficient. Obesity on the other hand is linked with elevated concentrations of leptin and this in turn could be a possibility for hyper secretion of LH in women with PCOS.

Many of the symptoms of PCOS are associated with psychological disturbance and any symptom may worsen a pre-existing tendency to psychological dysfunction. The menstrual cycle is commonly erratic in PCOS and periods when they occur are often heavy, prolonged and unpredictable. Furthermore in anovulatory women, estradiol acts on the endometrium without any opposition since there is absence of production of cyclical progesterone. This results in events of uneven menstruation, which can lead to endometrial hyperplasia and endometrial carcinoma.

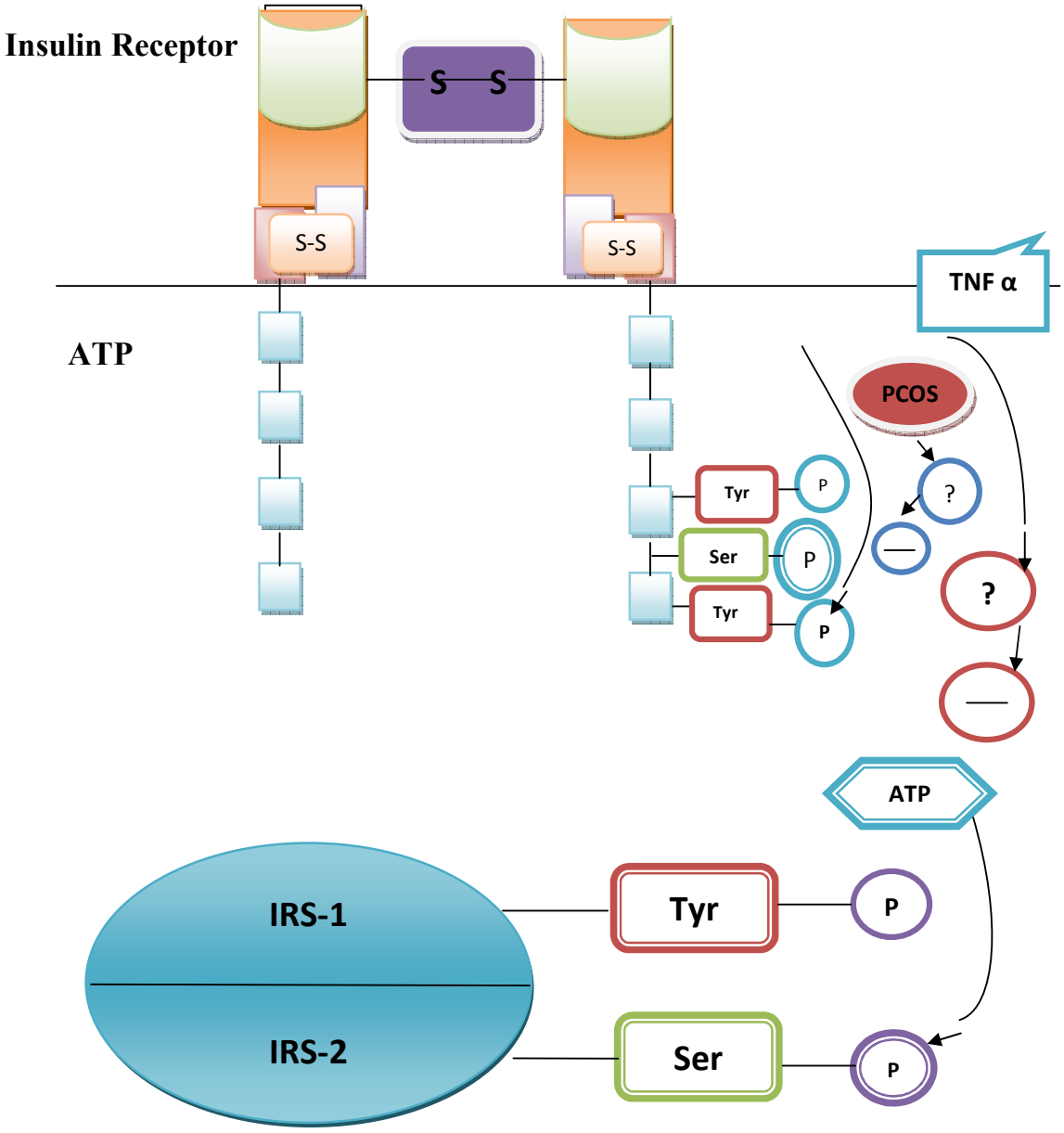
Menstrual dysfunction brings with it various psychological problems, in particular, mood changes, which are often described as being similar to premenstrual type symptoms. It is likely that **'hormonal imbalance'** or the erratic nature of the cycle may lead to altered mood and psychological distress[41].

Insulin resistance and its associated hyperinsulinemia leads to a sequence of events collectively called as insulin resistant syndrome. The sequence of events include cardiovascular disease, hypertension, polycystic ovary syndrome and non-alcoholic steato hepatitis[42]. Insulin resistant syndrome and diabetes are inter-related but are different entities. All insulin resistant individuals need not be diabetic, but develops diabetes only if he is not able to make up the degree of hyperinsulinemia needed to overcome the insulin resistance. Eventhough an insulin resistant individual need not be diabetic the risk of developing the consequences of insulin resistance syndrome are high. Patients with insulin resistance syndrome have increased concentrations of plasminogen activator inhibitor-1, C-reactive protein and increased white cell count. Hence these patients are at increased risk of developing PCOS.

Picture3:Insulin Resistance and its Complications



Picture 4: Insulin Signaling Pathway



IRS – Insulin Receptor Substrate

Tyr- Tyrosine

Ser- Serine

Receptor of human insulin is a **heterodimer** consists of two α, β- dimers. A disulfide bond links both these α, β- dimers. The α- subunit is extracellular and comprises the binding domain for receptor. The β- subunit is intracellular and it contains the intrinsic protein tyrosine kinase activity. When a ligand binds to receptor of insulin it causes conformational change i.e autophosphorylation of this receptor at tyrosine site and promote stimulation of its intrinsic kinase activity. In PCOS patients there is fall in insulin receptor autophosphorylation. This is because of increased basal autophosphorylation. Phospoaminoacid analysis demonstrated insulin-dependent receptor tyrosine phosphorylation was reduced and augmented insulin independent receptor serine phosporylation[43]. Serine phosphorylation of this receptor in initial phases of the insulin signaling pathway causes IR in PCOS women. Insulin independent serine phosphorylation of the insulin receptor in PCOS women is a sole condition of insulin action as further insulin- resistant states like obesity and non- insulin dependent diabetes mellitus [NIDDM] do not exhibit this abnormality[43, 44].

Low grade inflammation which is of **chronic** is related to obesity, IR syndromes such as polycystic ovary syndrome, and an increased risk of cardiovascular disease[45]. In obese patients enlargement of adipose tissue

causes adipocyte hypertrophy and hyperplasia and this huge adipocytes outshine the local supply of oxygen causing the cell hypoxia independently with stimulation of cellular stress pathway[46]. This leads to independent inflammation of the cells and cytokines and other pro-inflammatory signals to get released. As a fragment of this chronic inflammatory process, locally released chemokines draws pro-inflammatory macrophages into the adipose tissue. These then produce structures similar to crown round the huge lifeless or dying adipocytes. These tissue macrophages release cytokines which then trigger the inflammatory process in adjacent adipocytes, aggravating inflammation and insulin resistance. There is genetic basis for the inflammation observed in PCOS[47]. Variants in genes encoding several pro-inflammatory cytokines and their receptors associated with insulin resistance, obesity and/or diabetes have also been found to be associated with PCOS[48-54]. Variants in the gene encoding TNFα[49], type 2 TNF receptor and IL-6[51-53] and its signal transducer[54] have been reported in association with PCOS in European population. These findings are in conceptual agreement with a common evolutionary background for PCOS and metabolic disorders.

There is controversy regarding relevance of circulating inflammatory molecules because most pro-inflammatory mediators exert their effect in tissue in autocrine and paracrine fashion. Interleukin-6 is an endocrine cytokine produced by mononuclear cell and adipose tissue that is directly responsible for stimulating hepatic CRP synthesis[55-58]. CRP in turn has emerged as a major predictor of metabolic dysfunction in asymptomatic individuals, and is also produced by adipose tissue[59, 60]. Hence **C-reactive protein** is the most consistent circulating marker of low- grade chronic inflammation in PCOS.

Like C-reactive protein and IL-6, IL-18 is also a proinflammatory cytokine which promotes secretion of TNF-α[61], which furtherstimulates the production of IL-6. **Interleukin-6** controls the production of CRP in the liver. Serum **Interleukin-18** levels also correlate with waist-to-hip ratio [WHR] and fasting insulin levels. Hence raise in serum IL-18 is related to obesity and insulin resistance. Monocyte chemoattractant protein-1 [MCP-1] and Macrophage inflammatory protein-1 [MIP-1] are other proinflammatory chemokines elevated in polycystic ovary syndrome. Monocyte chemoattractant protein plays a major role in the development of atherosclerosis, while Macrophage inflammatory protein is also called as chemokine [c-c motif] ligand 3 [CCL-3] which

shows a significant role in recruitment and activation of leukocytes and predicts future cardiovascular events[62].

In addition to pro-inflammatory cytokines and chemokines there are also other markers of inflammation found to be elevated in women with polycystic ovarian syndrome. These include the WBCs, oxidative stress, advanced stress, advanced glycation end products, endothelial inflammation and infections.

The WBC[White blood cell] count is found to be elevated in polycystic ovarian syndrome women with significant increase in lymphocytes and monocytes. Elevation of WBC count in women with polycystic ovarian syndrome correlated with the insulin resistance.

Oxidative stress and inflammation are inter-related. Hence there is an association between oxidative stress and diabetes, obesity, metabolic syndrome and polycystic ovarian syndrome. Oxidative stress is identified by evaluating lipid peroxidation using erythrocyte malondialdehyde assay. In patients with polycystic ovarian syndrome there is increase in lipid peroxidation and this was directly proportionate to body mass index [BMI], insulin levels and blood pressure. In addition patients with polycystic ovarian syndrome also have decreased total antioxidant status, reduced haptoglobin a protein with antioxidant

properties. Hence oxidative stress is directly proportional to insulin resistance and reactive oxygen species production by mononuclear cells in regard to hyperglycemia which is higher in women with polycystic ovarian syndrome.

When there is a non- enzymatic reaction between reducing sugar and amino groups of proteins advanced glycation end products [AGEs] are generated. There is induction of oxidative stress when these advanced glycation end products act on signal transducing receptors [RAGE]. Recent studies demonstrate an elevation of AGE products in women with polycystic ovarian syndrome[63].

Chronic low grade inflammation is linked to endothelial inflammation and subsequently to endothelial dysfunction. Polycystic ovarian patients have endothelial dysfunction this in turn is resistant to insulin induced vasodilation. Also polycystic ovary syndrome is linked with various markers of dysfunction of endotheliun and inflammation including endothelin-1, soluble intercellular adhesion molecule-1 [sICAM-1], soluble vascular cell adhesion molecule-1 [sVCAM-1] and plasminogen activator inhibitor-1. Hence abnormal endothelial function in polycystic ovary syndrome is proportional to its insulin resistance.

There are few studies stating that there is an association between polycystic ovary syndrome and low grade chronic infections. Patients with oligomennorhea and hirsuitism show seropositivity to Chlamydia Pneumonia and Chlamydia trachomatis[64]. These patients also have elevated high sensitive- C reactive protein. H.Pylori infection was also more prevalent in polycystic ovary syndrome women than age matched controls[65].

The health magnitudes of this syndrome are a lifelong issue. The prevalence of impairment of glucose tolerance, insulin resistance and diabetes is raised considerably in polycystic ovary syndrome. Cardiovascular risk factors comprising high cholesterol levels, hyperandrogenemia, hypertension, markers of a prothrombotic state and inflammatory markers are increased in women with polycystic ovary syndrome. Altered vascular, endothelial function and increased incidence of metabolic syndrome are also noted in polycystic ovary syndrome.

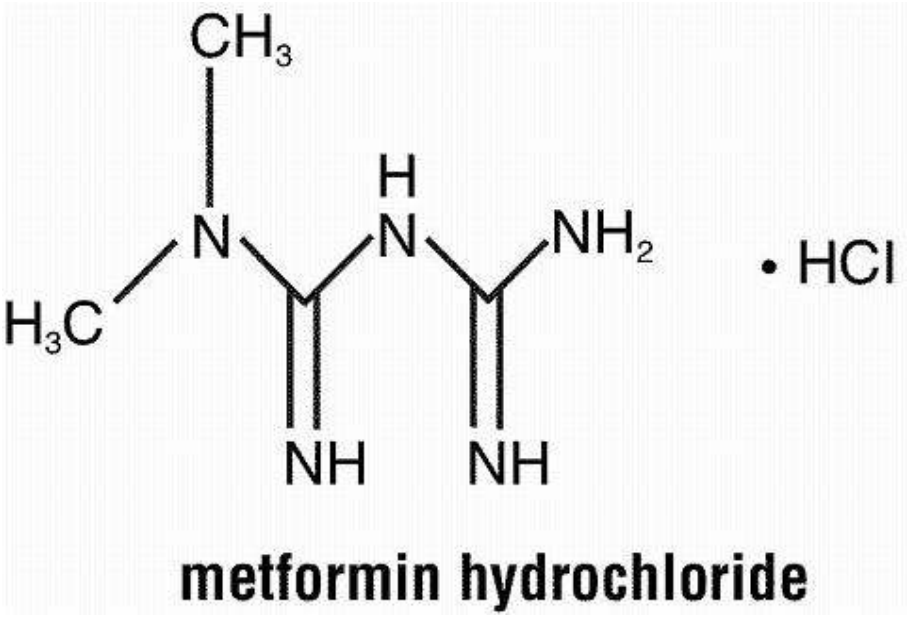
Numerous therapies have been projected for the treatment modalities of polycystic ovarian syndrome, but the ideal treatment for infertile women with polycystic ovarian syndrome has not yet been clear. Drug treatment comprises clomiphene citrate, insulin sensitizing agents, gonadotropins

and gonadotropin- releasing hormone (GnRH) analogs. Surgical treatments include laparoscopic ovarian drilling and the application of assisted reproduction techniques. Besides this loss of weight, diet and physical exercise have been shown to develop hyper insulinemia, menstruation abnormalities and ovulatory rates.

Polycystic ovary syndrome women develop menstruation irregularities owing to abnormal hormonal concentrations which in turn lead to the development of endometrial carcinoma, so in a women with menstrual irregularities it is more important to prevent development of endometrial hyperplasia than to regularize her menstrual cycles. Hence intermittent induction of menstruation using progestogens or intake of oral contraceptives either cyclically or continuously inhibits atypical proliferation of uterus. Combined oral contraceptives is utmost common treatment for the symptoms of polycystic ovary syndrome as they impede with actions of androgen and reduce androgen production, increased hepatic serum hepatocyte binding globulin synthesis, binding to androgen receptors competitively by some progestogens. Combined oral contraceptives have been revealed to decline insulin sensitivity, impair glucose tolerance and alter the lipid profile. Hence use of combined oral contraceptives alone for polycystic ovary syndrome make

them vulnerable to metabolic distresses. Treatment that combine the combined oral contraceptives with insulin sensitizers, antiandrogens, or both are developing with possible favorable effects on metabolic defects, particularly young females with polycystic ovary syndrome.

Metformin is a biguanide and is connected to guanidine and galegine. It is extracted from the plant Galega officinalis. It was in1922 metformin was first defined in works by Werner and Bell as a product in production of "N", "N"- dimethyl guanidine[66].



They found that dicyanodiamide was the precursor to guanidine derivatives. Dicyanodiamide when reacts with dimethyl ammonium chloride in acidic conditions for three to four hours, then dimethyldiguanide [i.e. metformin] is formed in good yields. Interest in metformin started in 1950s where metformin did not cause fall in the blood pressure and heart rate in animals not like related compounds. Garcia thought metformin to have bacteriostatic, antiviral, antimalarial, antipyretic and analgesic action. In 1954, Polish Pharmacologist Janusz Sipniewski in a series of articles was not able to demonstrate most of these effects, including lowered blood sugar[67].

Sterne was the first one who used metformin on humans for the treatment of diabetes. He published his results in 1957 and called the drug "Glucophage" [glucose eater]. Only in 1994 U.S food and drug administration [FDA] gave approval for the drug, even though it was approved in Canada in 1972[67].

Since Polycystic ovary syndrome is linked with insulin resistance, antidiabetic therapy has been prepared since late 1980s. It was in the University of The Andes where metformin use in polycystic ovary syndrome was first started. In United Kingdom, National Institute of Health and Clinical Excellence suggested in 2004 to give metformin for

women with polycystic ovary syndrome and body mass index above 25. This was prescribed for anovulation and infertility if further treatment options failed to yield the results. Metformin is not recommended as the first line of treatment for polycystic ovary syndrome by the United Kingdom and International Clinical Practice guidelines, except for women with glucose intolerance[68].

Metformin acts by decreasing hepatic glucose production and increasing hepatic glucose uptake. This has been implicated as a major contribution to glucose lowering effect. AMP-activated protein kinase (AMPK) affords a contender goal mediating useful metabolic possessions of metformin. AMPK has a wider part in metabolic processes which includes oxidation of fatty acid, glucose uptake by the muscle, glucose stimulated genes associated with hepatic lipogenesis. Thus metformin by activating AMPK controls type 2 diabetes mellitus[68].

Various dose regimens have been projected. For increasing patient's acceptance metformin is commenced at 500 mg every day as a single dose along with food. The dose is raised by 1000 mg after seven days and further it is again increased to 1500 mg. The dosage targeted is 1500–2550 mg/day which is given as 500 or 850 mg thrice daily. The effective response is perceived at the dose of 1000 mg once a day.

PCOS patients who do not respond to metformin at 1500 mg daily will positively respond to 2000 mg daily. Lactic acidosis is one of the adverse reaction noted in patients with renal failure, heart failure and sepsis.

Glueck et al showed that metformin has resulted in no main birth abnormalities and also no developmental defects in infants at the age of 3 and 6 months[69, 70]. One study compared the control group of women who was not treated with metformin and the treated group. They showed that the prevalence of diabetes during pregnancy in the metformin group was considerably less. In new PCOS, metformin compared with placebo shows improvement in ovulation rates. Metformin when compared with CC showed no advantage in ovulation, pregnancy, or live birth. In new PCOS, when metformin or clomiphene is given as single therapy they are more effective than the combination of metformin and clomiphene[71]. In Clomiphene Citrate resistant patients, it has no advantage in ovulation and live birth amounts over placebo as monotherapy. The mixture of metformin and Clomiphene citrate enriched ovulation and pregnancy than CC alone[72, 73]. Pre-treatment with Metformin increases the effectiveness of this drug in PCOS patients[74].

Beyond its effect on glucose metabolism metformin also plays a role in reduction of plasminogen activator inhibitor (PAI)-1, von Willebrand factor (vWF), smooth muscle cell (SMC) contractility via agonist induced increase in intracellular (Ca^{2+}) or a secondary increase in nitric oxide[75].

Metformin also plays a role in inflammation by decreasing inflammatory markers in plasma including soluble intercellular adhesion molecule, vascular cell adhesion molecule-1, macrophage migration inhibitory factor and C-reactive protein (CRP) in case of Polycystic ovary syndrome. IL-8 plays a dominant part in recruitment of monocytes and adhesion to endothelial cells in atherosclerosis and IL-6 plays a role in acute phase response. Metformin hinders IL-1β stimulated IL-6 and IL-8 manifestation exhibiting the mechanism through which metformin therapy attenuates inflammation. In smooth muscle cells of human in atherosclerotic plaques there is stimulation of multipotent pro-inflammatory transcription regulator. Metformin acts by inhibiting NF-κB activation hence modulates inflammatory response to IL-1β[76].

EL Mekkawi SF, ELHosseiny AS, et al. showed that there was a decrease in interleukin-6 and interleukin-18 level after metformin

treatment for three months in polycystic ovary syndrome patients. This shows metformin decreases the inflammatory markers and have beneficial effect on inflammatory process of PCOS[77].

Troglitazone is also an insulin-sensitizing drug which improves the ovulation and the pregnancy. But, because of its liver toxicity, it has been taken away from the market. Alternative medication in the similargroup, rosiglitazone (8 mg/day) which shows to augment impulsive and also clomiphene-stimulated ovulation in PCOS women[78]. **Pioglitazone** also seems to be in effect. But its safety is also questioned. Foetal safety has not been recognised for both rosiglitazone and pioglitazone which comes under pregnancy category C of US FDA 2 guidelines.

Tang et al recently reviewed the insulin-sensitizing drugs like metformin, rosiglitazone, pioglitazone, and d-chiro-inositol for PCOS females, oligo/amenorrhea, and subfertility. They finalised that metformin is beneficial in the pregnancy and also ovulation. Though, there is no proof that metformin rises birth rates when this is given as monotherapy or combined with clomiphene. Thus, the usage of metformin in improving fertility consequences in women with PCOS seems to be limited[79].

Even small amounts of FSH administered into the circulation along with FSH injections or with pulsatile GnRH or clomiphene citrate, is adept of stimulating ovulation and pregnancy in a huge number of women with PCOS with anovulation. It has been in effect in ovulation induction for PCOS women and considered to be the main line treatment of those patients. **Clomiphene citrate** is a partial oestrogen agonist. Clomiphene citrate is strictly related to the oestrogen chlorotrianisene. Absorption of this compound through oral route is efficient. Its half-life is 5–7 days and is excreted through urine mainly. It has strong binding to protein and enterohepatic circulation and is circulated to adipose tissues.

The estrogenic agonistic actions are well seen in animals with gonadal deficiency. It blocks the oestrogenic actions. It causes an increase in the production of gonadotropins and oestrogens by blocking the negative feedback effect of estradiol on the gonadotropins. The importance of this drug is seen on its efficiency to induce ovulation in oligomenorrhea or amenorrhea women and disorder of ovulation. Clomiphene blocks the feedback inhibitory stimulus of oestrogens on the hypothalamus, leading to a rush of gonadotropins, which causes ovulation.

Generally, one ovulation is stimulated by a single course of drug, and the patient must be treated subsequently till the pregnancy is reached, as

normal ovulatory action does not usually occur. The drug has no role in ovarian or pituitary failure patients. When clomiphene is given in a dose of 100 mg/d for 5 days, an increase in plasma LH and FSH is seen after many days. In ovulatory patients, there is an early rise followed by another rise of gonadotropin levels just preceding to ovulation. Careful monitoring of ovarian response with serial ultrasound scans is essential and in those who respond sensitively to 50mg, a lower dose of 25 mg may be used. A duration of three to six ovulatory cycles is usually adequate to distinguish whether pregnancy will be attained using clomiphene citrate, before going on to a multifaceted treatment, as around 75% of the pregnancies attained with clomiphene citrate happen in the first three cycles of treatment[80]. If the patient is ovulating, it is not essential to raise the dose even if conception has not resumed, and conception is anticipated to happen at a rate determined by influences like the patient's age. All women who are given with clomiphene should be cautiously observed with combined endocrine and ultra-sonography assessment of follicular growth and ovulation, for the reason of multiple pregnancy[81].

Ovulation occur in about 80% but then end result of pregnancy is only approximately 35-40% of patients who are given clomiphene[82].

Clomiphene citrate normalises LH and increase FSH secretion. This result in inducing follicle growth and ovulation. Various studies reported clomiphene to be the standard treatment for the patients of polycystic ovary syndrome for their induction of ovulation[83, 84]. A meta-analysis showed use of clomiphene citrate in PCOS women compared to placebo has six times more potency to result in pregnancy[83]. In practice, the usual regimen is an initial dose of 100 mg once a day from day 4 or 5 and this has no benefit by a daily dose of more than 150 mg which appears to considerably increase either the ovulation rate or follicular recruitment. This type of schedule will decrease the number of 'superfluous' cycles of therapy until those resistant to clomiphene are identified.

A prospective study on the follow-up of lean women with disorder of ovulation has reported more conception rates in patients responding to ovulation who are being treated with clomiphene. 50% achieved ovulation after three cycles and 75% after nine cycles of treatment[83].

Despite its efficacy some women do not ovulate and do not achieve pregnancy due to its adverse effects on endometrium like hyperandrogenemia, ovarian volume, menstrual irregularities and obesity. Also patients with increase basal LH have less chance to

respond to clomiphene[85]. Kousta et al showed treatment with clomiphene citrate for 167 patients showed a rate of 67.3% of conception over six months in patients who do not have any subfertility factors[86]. These patients were continued with twelve cycles of therapy. They described a multiple pregnancy rate of 11%, like to that referred in other series, and a miscarriage rate of 23.6%, with those who had miscarriage because of high LH levels immediately after clomiphene administration.

Shoham and colleagues observed the hormonal profiles in forty one women cured with clomiphene citrate of whom twenty eight ovulated[87]. In those who ovulated, 17 had normal patterns of hormone secretion and five of them conceived. 11 of them who showed abnormal patterns of hormonal profile, high levels of LH from day 9 until LH spur, along with premature luteinisation and high concentrations of estradiol throughout the cycle, did not conceive.

If pregnancy was not achieved even after 10-12 normal ovulatory cycles it is then apt to suggest the couple assisted conception. The addition of an ovulatory dose of human chorionic gonadotropin (hCG), 5000-10000 IU can be administered if the cause for non-ovulatory response is delayed LH surge or absent of it even in the occurrence of a well-

developed follicle. The repetitive administration of hCG at mid-cycle result in little enhancement of conception rates if given when follicle reaches a diameter of 18-24 mm.

Daily doses of dexamethasone, 0.5 mg at bedtime, as an aide to clomiphene citrate treatment, suppress the production of adrenal androgen and may stimulate receptiveness to clomiphene in former non-responders, commonly PCOS hyper androgenic women with increased levels of dehydroepiandrosterone sulphate (DHEAS) [88, 89]. But side effects are more with this method.

A systematic review by Misso M et al. shows that combination of Clomiphene Citrate and metformin are improved than placebo for increasing ovulation and pregnancy rates. But CC is superior to metformin for ovulation, pregnancy and live-birth rates, in PCOS patients with body mass index (BMI) >30. A combined CC and metformin is more efficacious than metformin alone or CC alone, depending on the BMI and CC sensitivity of the patient[90].

A Prospective trial was conducted to compare the ovulation and pregnancy rates in infertile CC alone and combined metformin and CC PCOS women for three cycles of escalating the dosage from 50 mg till 150 mg alone compared with CC and then another 150 mg for three

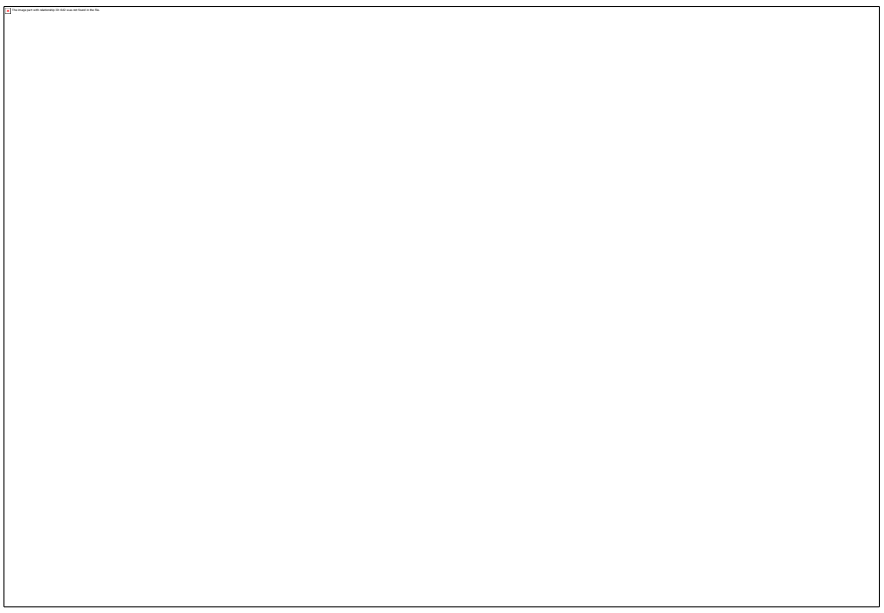
cycles. Another group were given the same amount of CC along with 1500mg of metformin. Up to six cycles ovulation or pregnancy was assessed by transvaginal sonography. Rate of ovulation and pregnancy in combination with metformin and CC was greater than CC alone. Moreover, the study showed metformin augmented the ovulatory rate in CC failures indicating increased responsiveness to CC[91].

Clomiphene citrate does not show any effect on inflammation in PCOS.

Menstrual irregularities are the most common symptom that occur in polycystic ovary syndrome. Amenorrhoea or oligomennorrhea is the primary complaint. In normal menstrual cycle oestrogen will be produced during ovulation and progesterone will also be secreted. The shedding of endometrium occurs if pregnancy doesn't occur. This is Secretory phase.

For this phase **progesterone** is necessary. In PCOS progesterone are not secreted and hence there will be no shedding of endometrium. If this is continued the lining of cells which are not shredded gets accumulated and can lead to endometrial hyperplasia. So regular shedding is necessary. This can occur by giving low dose of progestogens cyclically and this induces regular menstruation. It can be given every month during secretory phase alone in low dose. Progestin after entering into

the cell gets bind to progesterone receptors which are dispersed in the middle of the nucleus and the cytoplasm. The ligand-receptor complex gets fixed to a progesterone response element (PRE) to stimulate the transcription of gene. The specificity of reaction is available in the cell and also other cell-particular receptor co regulators and relating transcription factors. The progesterone-receptor complex forms a dimer formerly tying to DNA. Like the oestrogen receptor, it can form heterodimers and in addition to homo dimers between two isoforms, A and B. These isoforms are delivered by optional splicing of the same gene[92].



Progesterone has little impact on protein metabolism. It empowers lipoprotein lipase action and appears to favour fat deposition. The consequences on carbohydrate metabolism are more distinct.

Progesterone increments basal insulin levels and the insulin reaction to glucose. There is normally no difference in carbohydrate tolerance[92].

Receptor of the renal tubule, bringing about a reduction in Na^+ reabsorption. This prompts to a high production of aldosterone by the adrenal cortex (eg, in pregnancy). Progesterone rises body temperature in humans. The mechanism of this process is not known, but a change in the hypothalamus on the temperature-regulating centers has been recommended. Progesterone also changes the function of the respiratory centers. The ventilatory response to CO_2 is raised by progesterone but synthetic progestins with an ethinyl group don't have respiratory causes. This causes to an amountable decrease in arterial and alveolar PCO_2in the pregnancy and in the luteal phase of the menstrual cycle. In the brain, progesterone have depressant and hypnotic effects too.

In breast, it is the reason for the development of the alveolobular secretory components. It also involves the preovulatory LH spur and leads to the maturation and secretory alterations in the endometrium which occur after ovulation.

Regular treatment with progestin can cause breast engorgement, rise in body temperature, headache, depression, weight gain, irregular menstrual cycle, breakthrough bleeding. Severe cases can lead to amenorrhoea.

The frequency of menstrual cycles is less significant than the occurrence of endometrial hyperplasia. Intermittent induction of menstruation utmost usually by progestogens or oral contraceptives, either cyclically or continuously, inhibits abnormal uterine proliferation. Use of **combined oral contraceptives (COCs)** is the usual treatment for the symptoms of polycystic ovary syndrome. The COCs help to prevent recurrent anovulatory bleeding by providing a progestin and by suppressing ovarian hormones and adrenal androgen production. They also, indirectly, increase sex hormone–binding globulin (SHBG). SHBG binds to androgens and reduces their circulating concentrations. Combined oral contraceptives decreases insulin sensitivity, impairs glucose tolerance, and alters lipid profiles in healthy women, but apparently it do not affect the risk of diabetes mellitus or cardiovascular disease[92].

Combined oral contraceptive pills contain low dose oestrogens and progestin so that side effects are minimised. If the patient contain

hirsutism and acne more estrogenic preparation can be preferred. There are monophasic, biphasic and triphasic combination pills[92].

Monophasic pills contain no phasic increase or decrease in the oestrogen /progestin content during 21 days of pill administration. The first pill is taken on the fifth day after the start of the menses. Thereafter, one pill is to be taken each day consecutively for 21 days. Then, next seven days are "pill free period" and the next course starts again after the fifth day of menses. On repeating this schedule, the cycle becomes anovulatory (due to estrogenic effect) with a regular cyclic menstrual bleeding on withdrawal of the pill (due to progestogenic effect). Biphasic and triphasic pills mimic the hormonal changes during the menstrual cycle as physiologically as possible. Biphasic pills have a fixed dose of oestrogen for twenty one days but with increasing doses of progesterone during two successive phases i.e. from the day one to ten and eleven to twenty one. Next seven days are pill free days. Triphasic pills provide a bit higher dose of oestrogen at near mid cycle but increasing doses of progesterone for three successive phases i.e. for days one to six, seven to eleven and twelve to twenty one days.

The combination pills supress the ovulation by inhibiting the release of FSH and LH [mainly estrogenic effect]. High doses of oestrogen also

inhibit LH surge. The role of progesterone is to ensure withdrawal bleeding after stopping the medication. Normal ovulatory cycle is regained within one to three months of stopping the COC[92].

COCs can also be combined with **Cyproterone acetate (CPA)** which is an anti-androgen. This not only regulates menstrual cycle but also effective in reducing the symptoms of hyperandrogenism such as hirsutism and acne. This in turn prevents endometrial hyperplasia. Cyproterone acetate (CPA) act chiefly by meddling with target tissue binding of androgens to their intracellular receptor. They are used as monotherapy or as combined therapy with COCs for masculinizing signs that are not reactive to a single drug. Anti-androgens will improve sensitivity to insulin slightly in PCOS women[93]. They competitively blocks receptors of androgen intracellularly, but it has less activity than spironolactone. The fall in LH-dependent androgen synthesis is ascribed to the progestational activity of CPA and **ethinyl estradiol (EE)** that is given with it to deliver contraception and also blocks LH. Upto 50-90% patients a clinical effect in hirsutism if CPA has been taken regularly for 2-3 months. 35µg of EE and 2 mg of CPA is mostly combined. As PCOS is a progressive syndrome, at least up to the age of forty, it is necessary to treat the patient with EE and CPA. This combination can

reduce androgen levels distinctly and also their adverse reactions. It will make the disease 'on hold'. So that the fertility rate increases even when the drug is discontinued. Other contraceptive drugs given periodically will regularize the periods and should be substituted after six months with the higher dose EE/CPA combination-since there is a chance of high possibility of thromboembolism. Cyclical progesterone therapy (using the less androgenic progestin such as medroxyprogesterone acetate and dehydrogesterone) may also be used to induce regular menstruation. A progesterone releasing IUCD may also be used to provide endometrial protection[93].

Falsetti L and Passinetti E studied the effects of combination of 0.035 mg of ethinylestradiol and 2 mg of cyproterone acetate on lipid metabolism in women with polycystic ovary syndrome (PCOS). For this, 72 PCOS women were given with thirty six repetitive cycles of EE and CPA. Profiles of hormones, lipid and sugar were read before and after 12 and 36 cycles of treatment. The treatment repressed gonadotropin and androgen levels and improved the concentrations of sex hormone binding globulin. After 12 and 36 cycles a substantial raise in triglycerides, high density lipoprotein (HDL) cholesterol system, and apoprotein B levels were perceived. Insulin and plasma glucose levels

had no change. During management triglycerides, total cholesterol and apoprotein B levels were more than the control group. There were no change in concentrations of LDL-C and HDL-C in PCOS and in the control group. Lipid and lipoprotein alterations seen after treatment can be because of oestrogen dominance of the treatment[94].

Maturation of oocytes is the final process of an immature female egg cells before fertilisation either in normal or stimulated ovarian cycles. Gonadotropins are the next line treatment for fertility in anovulatory women with PCOS[95]. **Follicle Stimulating Hormone (FSH)** stimulates growth of follicles, ova development and production of oestrogens. **Luteinizing Hormone (LH)** stimulates preovulatory expansion of the ripe Graafian follicle and induces ovulation and then leutinization of the ruptured follicle and corpus luteum is sustained till the next menstrual cycle. Progesterone secretion occurs only under the influence of LH. GnRH action gonadotropins occurs via a G-protein coupled receptor which exerts its action by stimulating intracellular Ca2+ through hydrolysis of phophotidyl inositol phosphate[96]. The production of gonadotropins raises at puberty and is more in women compared to men. In the follicular phase, moderate levels of FSH and low levels of LH will be present. There is a mid-cycle spur of both, but more of LH, just

before ovulation, followed by subsequent decrease in the luteal phase. Inadequate gonadotropins secretion results in amenorrhoea and sterility in women. However excess production of gonadotropins in women causes polycystic ovaries[97]. The gonadotropins causes induction of ovulation, maintenance and incite ideal growth of follicle through a regulated injection of FSH, and attain a follicular size which is able to get fertilize. Gn doesn't put forth an antiestrogenic action peripherally. The major disadvantage of Gn is the formation of many follicles so that increases the ovarian hyper stimulation syndrome (OHSS) and multiple pregnancies. FSH injection requires skill and rigorous monitoring and also very expensive and needs more time[98]. Various therapy designs are employed like step-up, low-dose step-up, and step-down regimens. The ASRM vouch for low-dose gonadotropin protocols[99]. The step-up regimen starts with dosage from 37.5–50 IU/day which is minimum and this is being increased regarding the deficiency of follicular response. The response are monitored using ultrasound and then treatment is altered 1 week later if there is no follicular development with an increase of 50% every time as needed.

HCG is useful as a substitute in the LH spur which causes maturation of the oocyte, follicular rupture and corpus luteal production. The step-

down course commences with the extreme suggested dose, which is then decreased as a follicle response is attained. The dose is reduced 50% every time when the therapy is altered. Current reports described higher protection for patients using the step-up regimen[100].

In 2006, the ASRM implied severe control when blood estradiol concentrations crossed 2500 pictogram/mL at the time of induction[99]. Recent suggestions advise withholding hCG injection in the availability of greater than two follicles >16 millimetre or greater than one follicle >16 millimetre and two extra follicles >14 millimetre, or if serum estradiol concentrations are within 1000 and 2500 pictogram/mL, especially in female less than 38 years old who had no other infertility causes. So low-dose regimens ended in a monofollicular ovulation rate of ~70%, a pregnancy rate of 20% each cycle, and multiple live birth of 5.7% even then the incidence of multiple pregnancies was around <6% and OHSS was around<1%[101]. A maximum of six cycles with gonadotropins is suggested since no action even for six cycles suggest their presence of resistance.

It is mainly attempted to stimulate ovulation along clomiphene citrate when failed or during anovulation which can be because of polycystic ovaries.

The usually used dosage of hCG in IVF is 10,000 International Units but studies shows 5,000 International Units is sufficient to yield similar results[102]. These hormones act on women for their fertility and ovulation and not on inflammation.

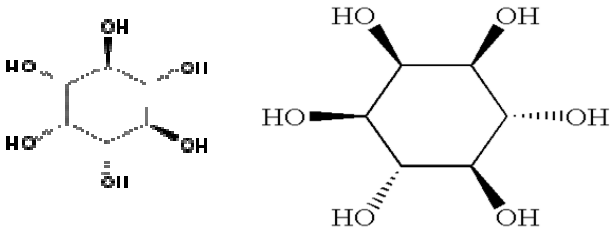
In 1850 Johannes Joseph Scherer[103]was the first man to isolate hexahydroxycyclohexane from muscle and named it Inositol as it belongs to the sugar family[104]. The hexahydroxycyclohexane structure has 9 different stereo isomers. Among them, **Myo-inositol** is the most distributed isomer in the body. It has both metabolic and functional importance. Myo-inositol is thought to be a prebiotic molecule[105] and inositol and inositol-derivatives has various roles in biological systems. Later inositol was found to be the main component of phytates which is the salts of inositol hexaphosphoric acid. In 1914, Anderson[106]showed the molecular structure of myo-inositol- 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate which is said also as phytic acid and confirmed by various modern analytical methods[107].

Inositol is found in foods like fruits and beans[108]. Myo-inositol is incorporated into the cell wall as phosphatidyl myo-inositol, the pioneer of inositol triphosphate which acts as a second messenger regulating the mechanism of hormones such as FSH, TSH and insulin. Addition to it,

inositol is an important component of the structural lipids[109]. After Scherer, many researchers started to study the role of inositol in different organs and tissues specifically its role in cell shape and oocyte fertility. In 1964, Eisenberg et al[110], and Eisenberg and Bolden[111] found that testes are rich of free inositol; later, Voglmayr and Amann[112], Lewin and Beer[113], and Ghafoorunissa[114] reported that the prostate, the epididymis and seminal vesicles contain a large amount of myo-inositol. The seminal fluid is one of the richest sources of inositol. The concentration of inositol in seminal fluid is almost three times higher than that found in plasma[115, 116].

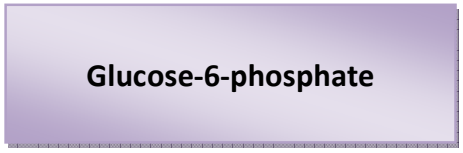
These primary findings gave the first indirect proof relating inositol based molecules to germ-cell (spermatozoa and oocytes) physiology. In 1974, Larner reported the presence of various intracellular chemical mediators of insulin, and hypothesized that, after the binding of insulin to its receptor; different intracellular pathways could be triggered according to the specific mediator involved[117]. In 1988 Larner et al[118] was drawn to a conclusion that the two inositol stereo isomers, Myo-inositol and D-chiro-inositol, are present. They are the chemical mediators of insulin. These both act through different mechanisms but

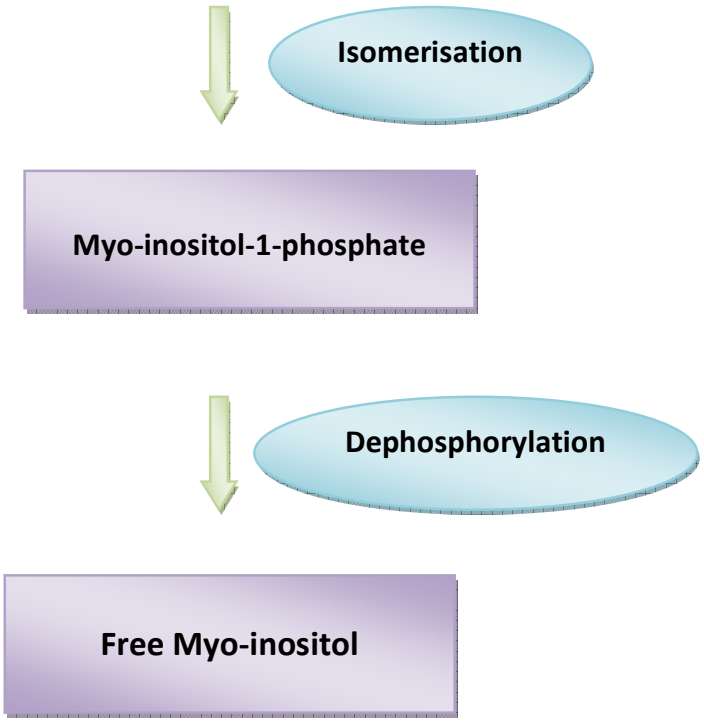
have same forms, changing only in the stereochemistry of having one hydroxyl group[119].



Natural sources for these inositols are endogenous biosynthesis and dietary intake. Myo-inositol is produced from glucose-6-phosphate in two steps. First, glucose-6-phosphate isomerizes to myo-inositol-1-phosphate, which is dephosphorylated by an inositol monophosphatase enzyme giving free myo-inositol[120].

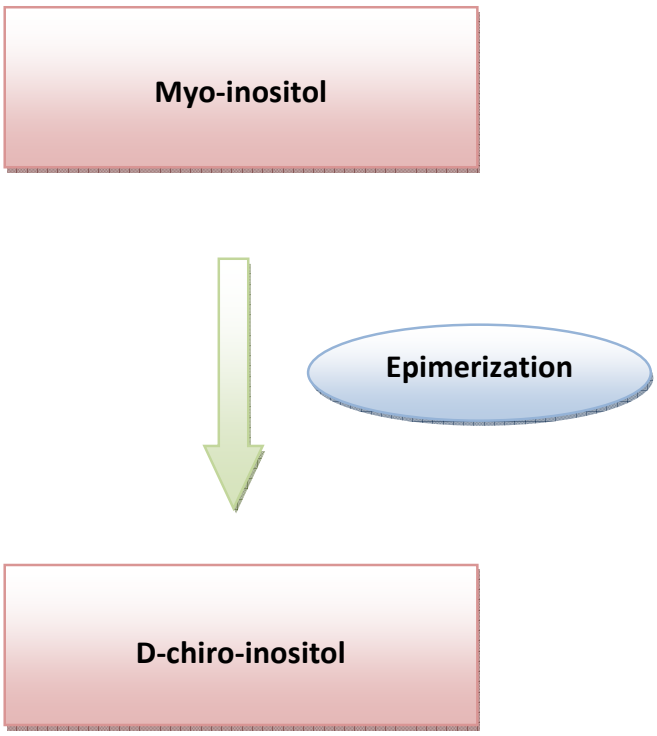
Picture 5: Formation of Myo-inositol





In vivo, **D-chiro-inositol** is synthesized by an epimerase that converts myo-inositol into D-chiro-inositol.

Picture 6: Conversion of Myo-inositol to D-chiro-inositol



Larner first studied a decreased D-chiro-inositol content in urine as well as tissues of human subjects and animals with type 2 diabetes[119, 121]. He postulated that the myo-inositol when converted to D-chiro-inositol requires stimulation of insulin[122]. This conversion is decreased in the insulin sensitive tissues like fat, liver, muscles of rats[123].

PCOS pathogenesis is linked to insulin resistance and/or compensatory hyperinsulinemia irrespective of their BMI whether they are lean or obese. The impairment in the insulin signaling in PCOS could be due to

a defect in the inositol phosphoglycans (IPGs) second messenger pathway[119]opened a new angle in the clinical management of PCOS. IPGs have a role in stimulating enzymes that regulate glucose metabolism[124, 125]. In PCOS women, a defect in tissue availability or altered metabolism of inositol or IPGs mediators may contribute to insulin resistance[126]. In 1999, Nestler JE et al. published Ovulatory and metabolic actions of D-chiro-inositol in the polycystic ovary syndrome assessed steroids level in serum and oral glucose tolerance tests before and after the oral administration of 1200 mg of D-chiro-inositol or placebo for six to eight weeks in 44 obese PCOS patients once daily. The results proved that PCOS patients who received D-chiro-inositol has improved insulin sensitivity and serum free testosterone levels were reduced than the placebo group. In addition to it, diastolic and systolic blood pressure, and plasma triglyceride levels were decreased in patients treated with D-chiro-inositol. Ovulation occurred in 19 out of 22 women (86%) who received D-chiro-inositol , as compared to 6 out of 22 (27%) in the placebo group[127].

In 2002, Nestler and Allan reported a clinical study in which they saw whether administration of D-chiro-inositol would affect the levels of circulating insulin and androgens, and the occurrence of ovulation in

lean PCOS patients. These results confirmed earlier findings which showed in lean PCOS women also, D-chiro-inositol reduces serum insulin and androgen and also improves PCOS-associated metabolic abnormalities (increased blood pressure and hypertriglyceridemia)[128].

Chiu et al in 1992 published a study which showed an increased concentration of inositol in patients with successful IVF pregnancies. This indicates the involvement of inositol in the early phase of in vitro IVF and also for the maintenance of normal development of embryo. They also reported that patients who had successful IVF pregnancies and also those who had high levels of myo-inositol showed the development of the embryos with higher number of somities[129]. Same group of authors[130] after ten years conducted a study to examine whether the content of myo-inositol in human follicular fluid was associated with good oocyte quality. For this fifty three patients who had been treated with IVF were recruited and their follicular fluid and serum were collected. Then they have been divided into two groups with group A consisting follicular fluid with matured and fertilized oocytes and group B has immature follicles and unfertilized oocytes. There was a statistical significance of correlation between the levels of myo-inositol in the follicular fluid and the quality of oocytes. This showed greater follicular

concentration of myo-inositol is associated with greater follicular maturity. So concentration of myo-inositol is a quality marker for evaluation for oocytes.

Unfer et al. conducted a study on myo-inositol administration in PCOS patients. Twenty five patients participated in this study and they were given myo-inositol and folic acid twice daily. Their ovulatory activity was seen through sonography and hormonal profile, number of spontaneous menstrual cycles and pregnancies were noted. The results obtained showed the efficacy of myo-inositol in simultaneous ovarian activity and fertility in PCOS patients[131].

Following this study many authors[132, 133]reported daily intake of myo-inositol improves hormonal profile, restores ovulation, and induces regular menses in PCOS patients who are both lean and obese.

Rizzo et al. reported the efficacy of myo-inositol and folic acid in fertility improvement in PCOS patients compared to Myo-inositol plus folic acid and melatonin. Unfer et al. again showed there was no difference in myo-inositol and D-chiro-inositol treated groups on the number of oocytes but mature oocyte number was increased and the immature oocytes got decreased in myo-inositol group[134].

In patients with PCOS there was a myo-inositol depletion which resulted in poor oocytes[135]. This myo-inositol depletion result in impairment of FSH signaling in PCOS patients[136]. Various studies reported shows the need of rFSH got decreased during IVF cycles when myo-inositol supplemented three months before the stimulation of ovary[134, 135,137].

Isabella and Raffone[140] showed increase in D-chiro-inositol will produce ovary toxicity which is characterized by poor quality of oocyte and reduced response of ovary to FSH. Another study by Galetta M et al. showed healthy volunteers had high myo-inositol concentration and low D-chiro-inositol concentration. But PCOS patients had opposite ratio. This is called "DCI paradox"[141].

Nowadays combined treatment of D-chiro-inositol and myo-inositol are also being given. Minnozi et al. showed the combined therapy of myo-inositol and D-chiro-inositol was able to improve the metabolic consequences of PCOS women, thus decreasing the cardiovascular risk factors. In this study twenty obese PCOS patients were taken and their lipid profile such as HDL, LDL, Triglycerides were assessed before and after six months of treatment with combination therapy. Changes in body mass index, waist-hip ratio, body fat percentage, blood pressure

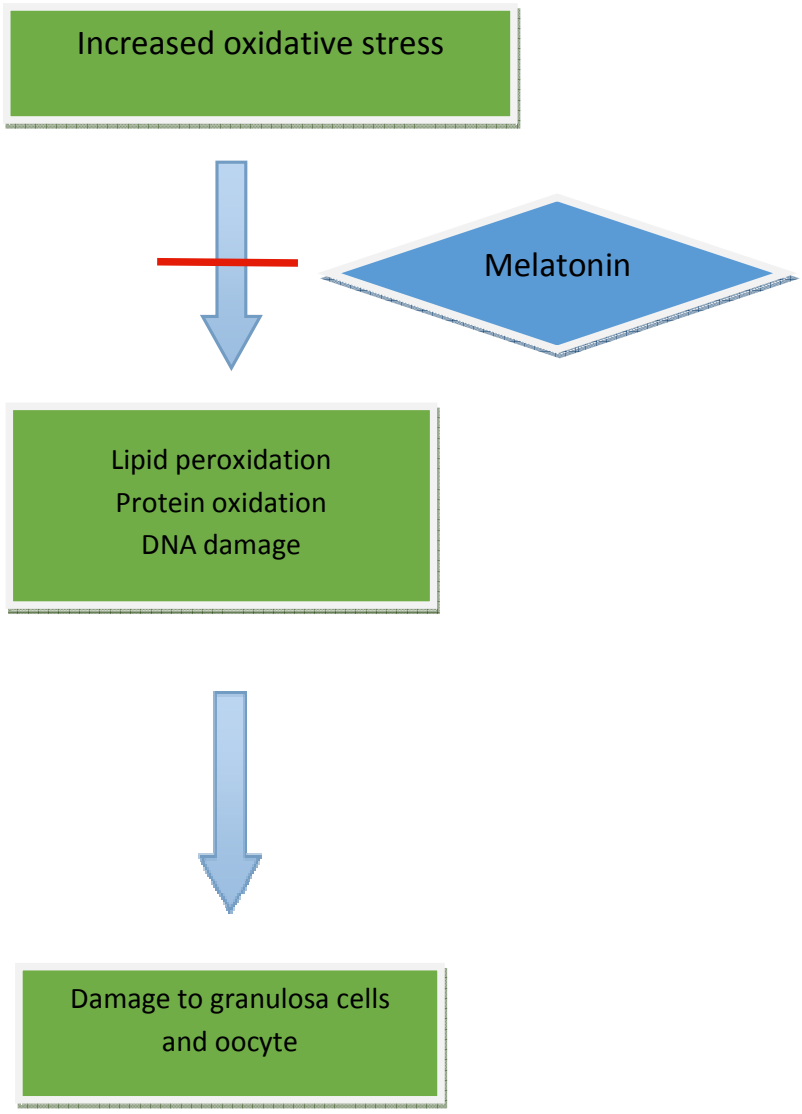
and HOMA-IR were evaluated. There was a strong increase in HDL levels and decrease in LDL and Triglycerides levels. Reduction in HOMA-IR was also seen concluding that the combination therapy improves the metabolic profile[142].

N-acetyl cysteine(NAC) acts by inhibiting the stress of oxidation and preventing the increased glucose levels which stimulates the resistance of insulin. A study by Fulghesu et al.[143] illustrated the patients with PCOS who had increased insulin levels when treated with N-acetyl cysteine, treatment with NAC resulted in progress of insulin sensitivity in addition to marked decrease in concentrations of testosterone and lipids. Kilic-Okman and Kucuk[144]has showed NAC decreases serum testosterone and insulin levels and improved homocysteine in addition to lipids in PCOS patients. Study by Rizk et al[145]. reported the combination of CC and NAC rises the rates of both ovulation and pregnancy in infertile CC-resistant patients infertile. N-acetyl cysteine plus clomiphene citrate when compared with clomiphene citrate alone showed improved follicular growth. Thus emphasizing that N-acetyl cysteine is effective in inducing and maturing the oocytes. A study by Saghar Salehpour et al[146]. reported that N-acetyl cysteine induces enhancement in profile of lipids, hormones, rate of ovulation, and as a

result the health outcomes of PCOS women. This is via the block stress of oxidation in addition to increasing the sensitivity of insulin in the tissues peripherally. The adverse drug events were very less due to NAC and hence it is taken into consideration for treatment of PCOS women.

As described earlier it is not only the anovulation but also decrease in the quality of oocyte and embryo were the reason for infertility in PCOS women. Poor oocyte quality is due to ROS-induced oxidative stress. Raise in lipid peroxidation products in serum as well as in follicular fluids were studied by various authors [147,148]. Sun C.L.et al showed apoptotic granulosa cells is more in PCOS females[149].

Picture 7: Mechanism of action of Melatonin



The major enzymatic metabolite of melatonin 6-sulfatoxymelatonin levels in urine is more in PCOS women than the female without PCOS[150]. LH production, and LH reaction to GnRH were enhanced due to elevated melatonin levels[150]. This high levels of **melatonin** may be due to feedback action to the lack of melatonin in the ovary. With this current understanding of actions melatonin may be a potential treatment for PCOS in the future.

Statins are the blocks of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase. It is the rate limiting process in the synthesis of cholesterol pathway. They strongly inhibit the secretion of cholesterol which is a substrate for testosterone as well as improves hyperandrogenemia. Apart from this they limit the mechanisms of insulin and IGF-1 on the ovary. This is done with the help of reducing N-linked glycosylation so that there will be maturation of insulin and Type 1 IGF-1 receptors. Not only this they also reduce isoprenylation of small GTPases such as Ras and Rac which are the mediators of insulin signaling pathway[151].

Statins blocks the mevalonate pathway which lead to abolition of the actions of increase of insulin levels on the proliferation and

steroidogenesis of thecal cells. They also inhibit the oxidative stress which causes increase in proliferation of cells, steroidogenesis and insulin resistance. So these two roles result statins to have a better effect in the PCOS and its effect on the cardiovascular mortality and morbidity.

Laparoscopic ovarian drilling is the surgical method in women with clomiphene-resistant PCOS and who persistently hyper secrete LH. After the surgery actions of ovary is restored and concentrations of LH and testosterone level fall[152]. Hence surgery helps to bring down the levels of LH and androgen even in patients with elevated LH concentrations. So laparoscopic ovarian drilling is more effective in patients with menstrual irregularities and increases the fertility rate in women who come to infertility clinics [153].

Even though these many drugs and modalities of treatment were available for PCOS, only few drugs targeted in reduction of inflammation. There were various studies, as we quoted earlier, demonstrating the effect of inflammation on PCOS. These studies were all done in other parts of the world. There were very less number of studies done in India. So we proposed to study the status of inflammation on PCOS in Indian population in particular South Indian

population. There were also as we said earlier studies on drugs targeting on inflammation in PCOS. These studies mainly spoke about metformin and their anti-inflammatory properties. There were no studies demonstrating the anti-inflammatory effect of other drugs used to treat PCOS in practise.

MATERIALS AND METHODS

This study was a **prospective cross sectional study**. The study population was from all the patients who attended the outpatient Department of Obstetrics and Gynecology in PSGIMSR Hospital, Coimbatore. We included all the PCOS patients according to Rotterdam consensus criteria with age group 18-45 years and excluded those patients presenting with a history of inflammatory diseases or intake of inflammatory drugs and also pregnant women. Healthy Volunteers of age group 18- 45 years women with normal reproductive cycle were included and those who present with history of inflammatory drugs or diseases are excluded. Further those who have hypertension or diabetes are also excluded from the healthy volunteers.

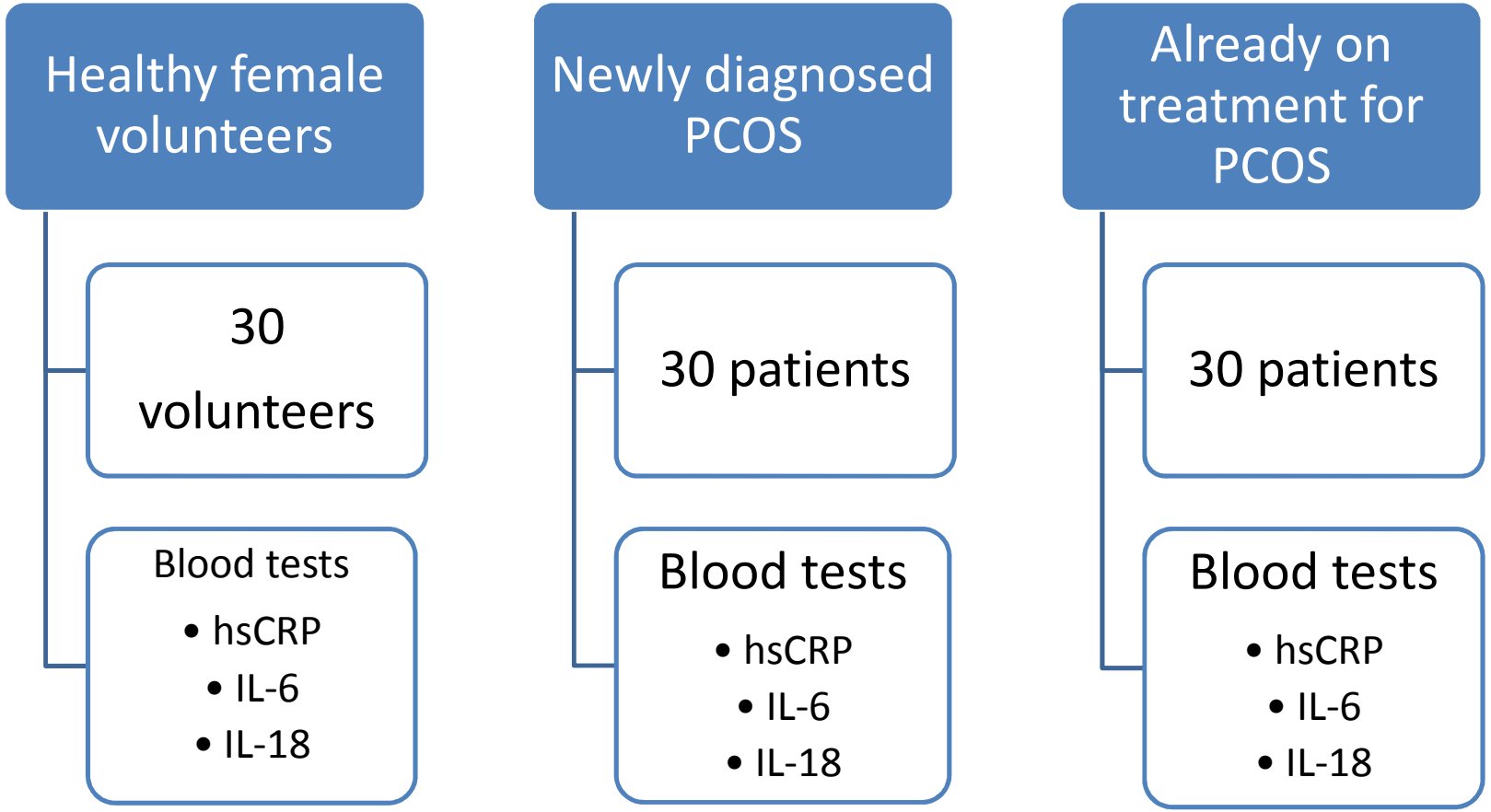
The overview of the study design is given in the figure below. The patients were divided into three groups.

Group 1: Healthy volunteers

Group 2: Newly diagnosed PCOS

Group 3: Already on treatment for PCOS

Sample size estimated according to previous literature search.30 participants in each group was taken.


Healthy female volunteers
30 volunteers
Blood tests
• hsCRP
• IL-6
• IL-18
Newly diagnosed PCOS
30 patients
Blood tests
• hsCRP
• IL-6
• IL-18
Already on treatment for PCOS
30 patients
Blood tests
• hsCRP
• IL-6
• IL-18

The study design was submitted to the Institute Human Ethics Committee (IHEC) stating its purpose preceding the study. The details and the usefulness of the study design were described to every participant individually and clarified their doubts before getting the informed consent forms. The informed consents forms provided to the participants were either in English or in Tamil. The participants, who gave written informed consent, came under the inclusion criteria were enrolled for the study. Healthy volunteers were also given the informed consent. The copy of consent forms were attached in the annexure. The patients who declined to give informed consent were also excluded from the study.

PCOS patients were selected according to Rotterdam Consensus Criteria 2003 which is given below.

- *Polycystic ovaries(12 or more follicles in each ovary, each follicle measuring 2-9 mm in diameter and/or ovarian volume > 10 ml, one polycystic ovary is sufficient for diagnosis)*

- *Oligo-/anovulation; clinical diagnosed as Oligo-/amenorrhea (menstrual cycles longer than 35 days or less than 10 menstruation/year)*

- *Hyperandrogenism (clinical or biochemical)*

Basic demographic data like name, age and anthropometric measurements of Height, weight, body mass index were recorded. History of the patient, any co morbidities was noted. If the patient is on already treated group then their treatment history were noted.

Our study was to observe if there were abnormalities in High sensitivity C - reactive protein, Interleukin-6 and Interleukin-18 in PCOS patients and to compare those with the healthy volunteers.

Sample for High sensitivity C - reactive protein (hs-CRP) was collected in **red topped vacutainer** which is a tube without any anticoagulant. The blood samples for Interleukin -6 (IL-6) and Interleukin -18 (IL-18) were collected in **yellow topped vacutainer** which had acid citrate dextrose as anticoagulant.

The high sensitivity CRP was tested in the Clinical Biochemistry lab using the **Cobas Integra C - reactive protein (Latex)**. This machine works by the methodology of "**Particle enhanced turbidimetric assay".** When Human CRP is applied over the monoclonal anti-CRP antibodies, it agglutinates due to exposure of latex particles. The precipitate is determined turbidimetrically at 552nm.Sample collected

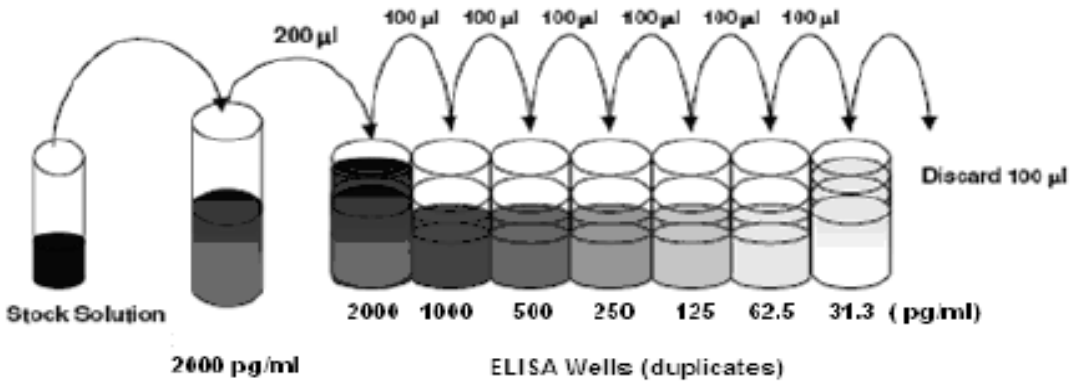
was centrifuged and the serum was used for analyses. The analyzer automatically calculates the analyte concentration of each sample. The value <0.05mg/dl was considered to be normal.

The Interleukin-6 was estimated in the Pharmacology laboratory using **Krishgen Biosystems,** imported from California, USA. The IL-6 enzyme immunoassays are programmed for measuring the quantity of human interleukin-6(IL-6). Samples and other requirements used for quantification are coated in microtiter plate which are previously coated with first monoclonal Ab, anti IL-6 in the presence of second Ab linked to streptavidin Horseradish peroxidase (streptavidin-HRP). Later when the incubation period is over these wells are washed and with the help of a colored substrate the enzymatic activity is detected. This color is proportional to the Interleukin-6 concentration in the serum sample.

Serum was obtained by allowing the blood to **clot** for 30 minutes and by **centrifuging** it for 10 minutes at 1000 x g. Then they were stored. Then the serum was separated carefully from clot in a separate plastic tubes and stored at <-20 degree C.

The kit contains a plate with ready to use 96 wells. The reagents were prepared for assaying IL-6. 5 ml of **20X wash buffer** was added to 95 ml and 1 ml of **5X Assay diluents** to 4 ml of distilled water. **25**

microliters of Detection Ab to 9975 microliters of 1X Assay Diluents to get a final volume to 10 ml and **50 microliters of streptavidin-HRP** to 9950 microliters of 1X Assay Diluents to get final volume to 10 ml were added. **Recombinant protein** was diluted by adding 20 microliters of std. solution in 10 ml of 1X Assay Diluents and top standard solution 2000pg/ml was prepared. Six two-fold serial dilutions were prepared from the top standard solution 2000picogram/ml such that concentrations are 1000picogram/ml, 500picogram/ml, 250picogram/ml, 125picogram/ml, 62.5picogram/ml, 31.3picogram/ml and 1X assay diluents serve as zero standard.



100µl/well of standards and samples were added to the plate. After sealing it was incubated at 37 degree C for 1 hour and 30 minutes.The wells were washed **at least for 4 times** using the wash buffer 1X and the

plate was turned upside-down and tapped firmly onto a clean absorbent paper.100µl of diluted Detection Ab was added to all wells and the plate was kept for incubation at 37 degree c for 90 minutes. Again the wells were washed using wash buffer 1X and 100 microliters of diluted streptavidin-HRP was added to each well after which the plate was sealed and incubated at 37 degree c for 30 mins. Wells were washed for four times following which it was soaked in wash solution for 30 to 60 seconds in between every wash to minimize the background error. Finally, freshly prepared **TMB substrate** 100 microliters was added to each well and was kept in the dark for 15 to 30 minutes. The wells showing positive reaction turned blue in color after which the reaction was stopped by adding 100 microliters of stop solution.

The plate was then inserted into an **ELISA reader** and the absorbance read at 450 nm within 30 minutes of adding stop solution. ELISA reader is a **Bio-Rad system** which is attached to a computer which has curve fitting software. The unknown concentrations of interleukin-6 were then identified by plotting the graph.

The Interleukin-18 was assessed in the Pharmacology laboratory using **Krishgen Biosystems,**imported from California, USA. This ELISA kit is based on the principle of double-antibody sandwich ELISA technique

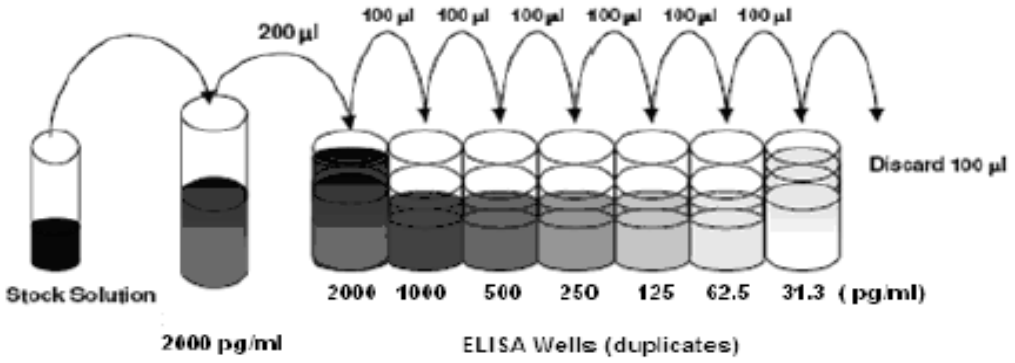
to detect Interleukin 18 (IL-18) in body fluids. The kit uses a double-antibody sandwich enzyme-linked immuno-sorbent assay (ELISA) to determine the amount of Human Interleukin 18 (IL-18) in a biological sample. Samples are added to micro-plate wells pre-coated with Human Interleukin 18 (IL-18) antibody. After incubation and washing, Human Interleukin 18 (IL-18) detection antibodies labeled with biotin is added. After incubation and washing Streptavidin-HRP is added to form immune complex, followed by incubation and washing to remove the uncomplexed enzymes. Then chromogenic TMB substrate solution is added, the color of the liquid changes to blue. Stop HRP enzyme reaction by addition of acid stop solution, when color changes to yellow. The intensity of color developed is proportional to the concentration of the Human Interleukin 18 (IL-18) present in the sample and read at 450nm using a plate reader.

Serum was obtained by allowing the blood to **clot** for 30 minutes and by **centrifuging** it for 10 minutes at 1000 x g. Then they were stored. Then the serum was separated carefully from clot in a separate plastic tubes and stored at <-20 degree C.

The kit contains a plate with ready to use 96 wells. The reagents were prepared for assaying IL-6. 5 ml of **20X wash buffer** was added to 95

ml and 1 ml of **5X Assay diluents** to 4 ml of distilled water. **25 microliters of Detection Ab** to 4975 microliters of 1X Assay Diluents to get a final volume to 10 ml and **50 microliters of streptavidin-HRP** to 9950microliters of 1X Assay Diluents to get final volume to 10 ml were added. **Recombinant protein** was diluted by adding 20 microliters of std. solution in 10 ml of 1X Assay Diluents and top standard solution 2000pg/ml was prepared.

Six two-fold serial dilutions were prepared from the top standard solution 2000picogram/ml such that concentrations are 1000picogram/ml, 500picogram/ml, 250picogram/ml, 125picogram/ml, 62.5picogram/ml, 31.3picogram/ml and 1X assay diluents serve as zero standard.



100µl/well of standards and samples were added to the plate. After sealing it was incubated at room temperature for 90 minutes.The wells were washed **at least for 4 times** using the wash buffer 1X and the plate was turned upside-down and tapped firmly onto a clean absorbent paper.100µl of diluted Detection Ab was added to all wells and the plate was kept for incubation at room temperature for 90 minutes. Again the wells were washed using wash buffer 1X and 50 microliters of diluted streptavidin-HRP was added to each well after which the plate was sealed and incubated at room temperature for 30 mins. Wells were washed for four times following which it was soaked in wash solution for 30 to 60 seconds in between every wash to minimize the background error. Finally, freshly prepared **TMB substrate** 100 microliters was added to each well and was kept in the dark for 15minutes. The wells showing positive reaction turned blue in color after which the reaction was stopped by adding 100 microliters of stop solution.

The plate was then inserted into an **ELISA reader** and the absorbance read at 450 nm within 30 minutes of adding stop solution. ELISA reader is a **Bio-Rad system** which is attached to a computer which has curve fitting software. The unknown concentrations of interleukin-6 were then identified by plotting the graph.

Statistical Analysis:

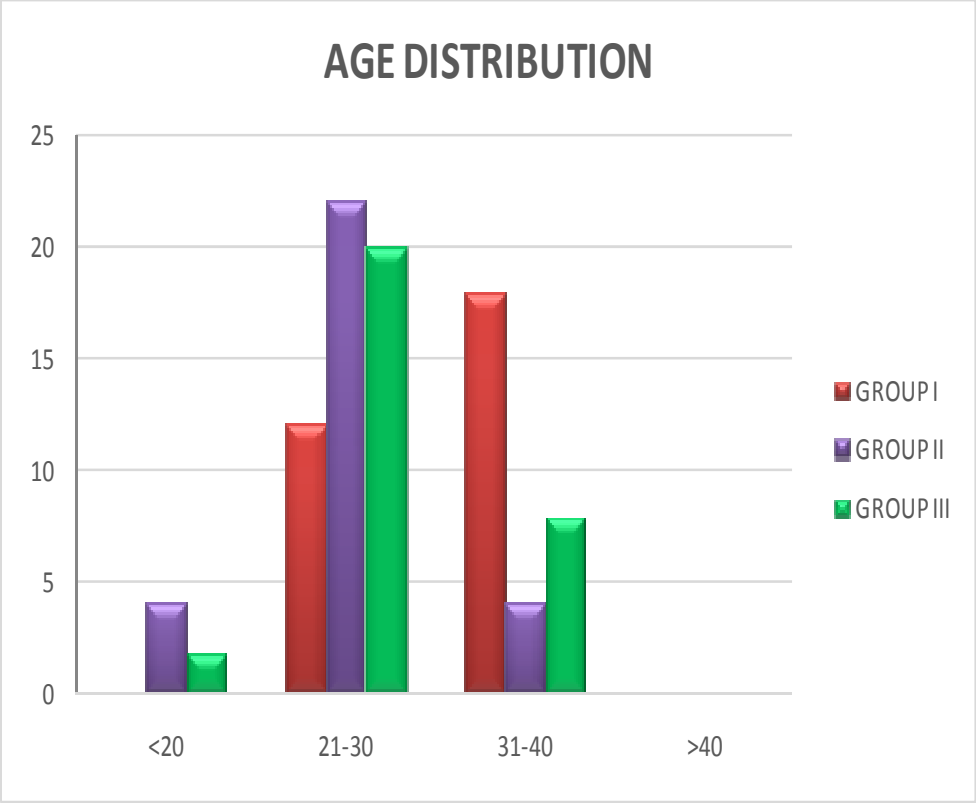
Statistical analysis were performed by using **SPSS software version 19**. The results were analyzed using **one way ANOVA** and post hoc analysis was done using **LSD**.

RESULTS

We included the patients who attended Obstetrics and Gynecology department and diagnosed to have PCOS. Their history when asked was more likely of amenorrhea or infertility. Of these, regarding infertility patients when history was asked it was found those patients have history of oligo/amenorrhea. Other patients were excluded.

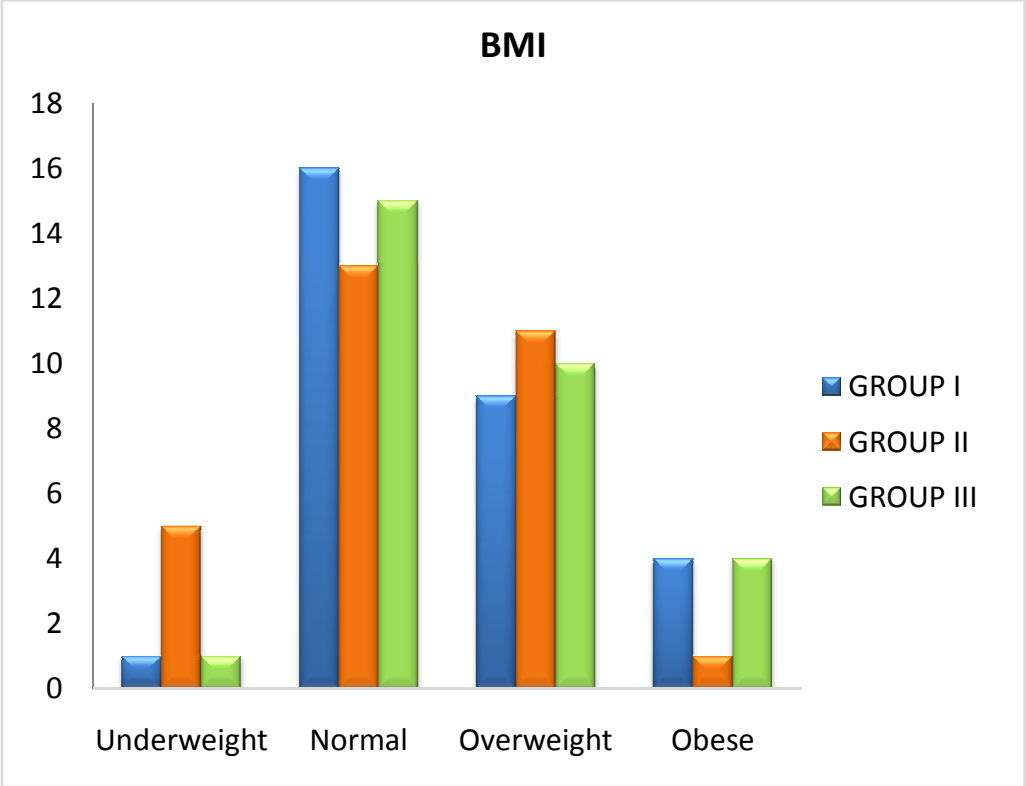
The statistical analysis done was **One-way ANOVA** and **post hoc LSD** using SPSS software version 19.

Figure 1 shows the age distribution of the study population



There were no patients above 40 years of age. 73.3% of newly diagnosed patients and 66.67% of already diagnosed patients are in the 3rd decade.

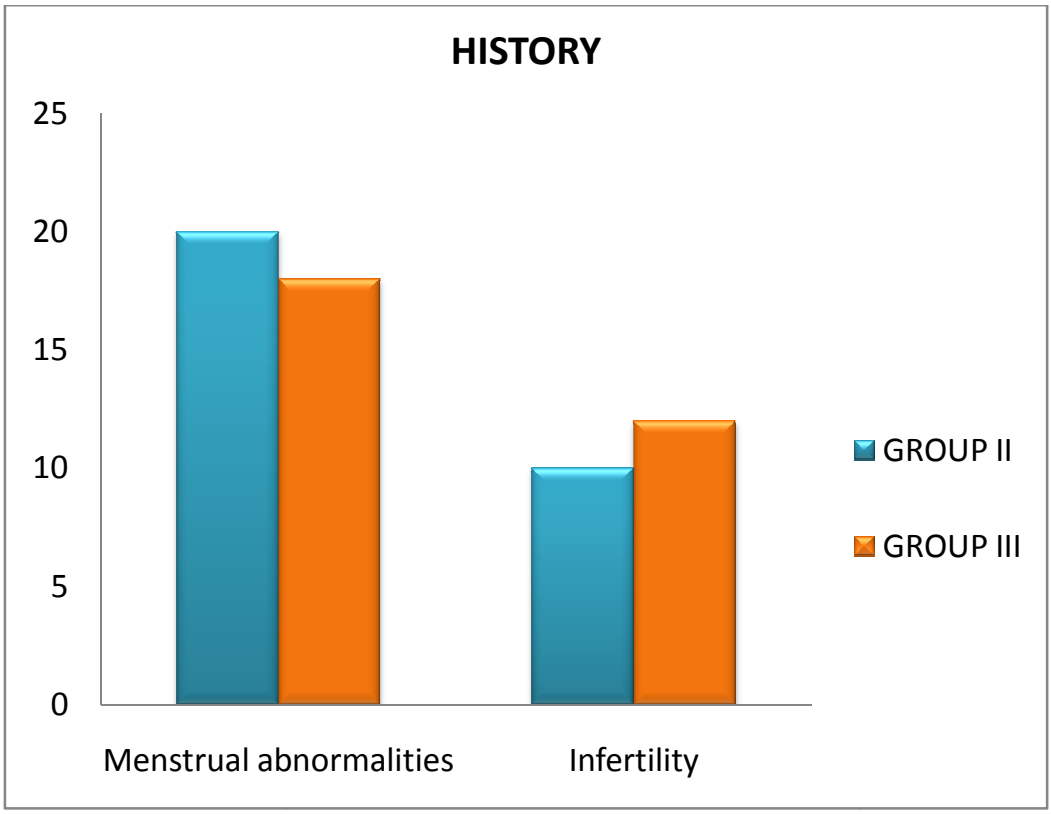
Figure 2 represents the Body Mass Index of the study population



43.33% of newly diagnosed and 50% of already diagnosed and 53.33% of normal healthy volunteers were normal. 36.67% of newly diagnosed and 33.33% of already diagnosed and 30% of normal healthy volunteers were overweight. 13.33% of normal and already diagnosed patients were obese and only 3.33% of newly diagnosed patients were obese.

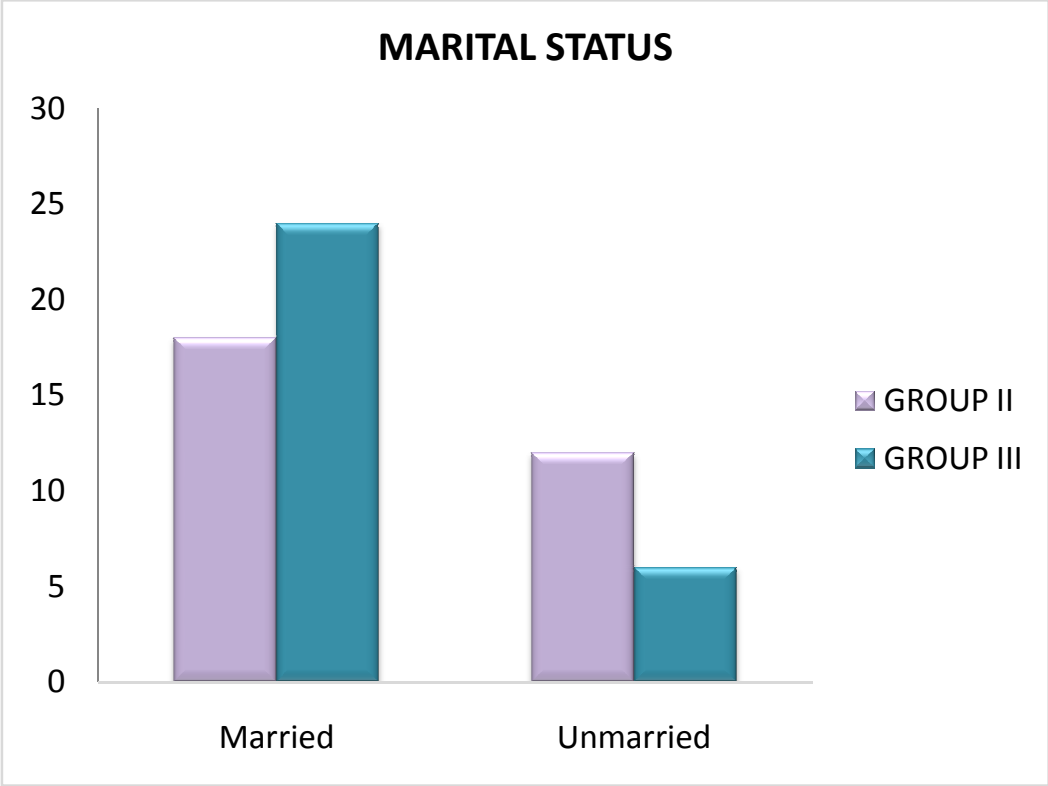
Figure 3 represents the history of the patients

The patients attended the outpatient department either for menstrual abnormality or infertility treatment.



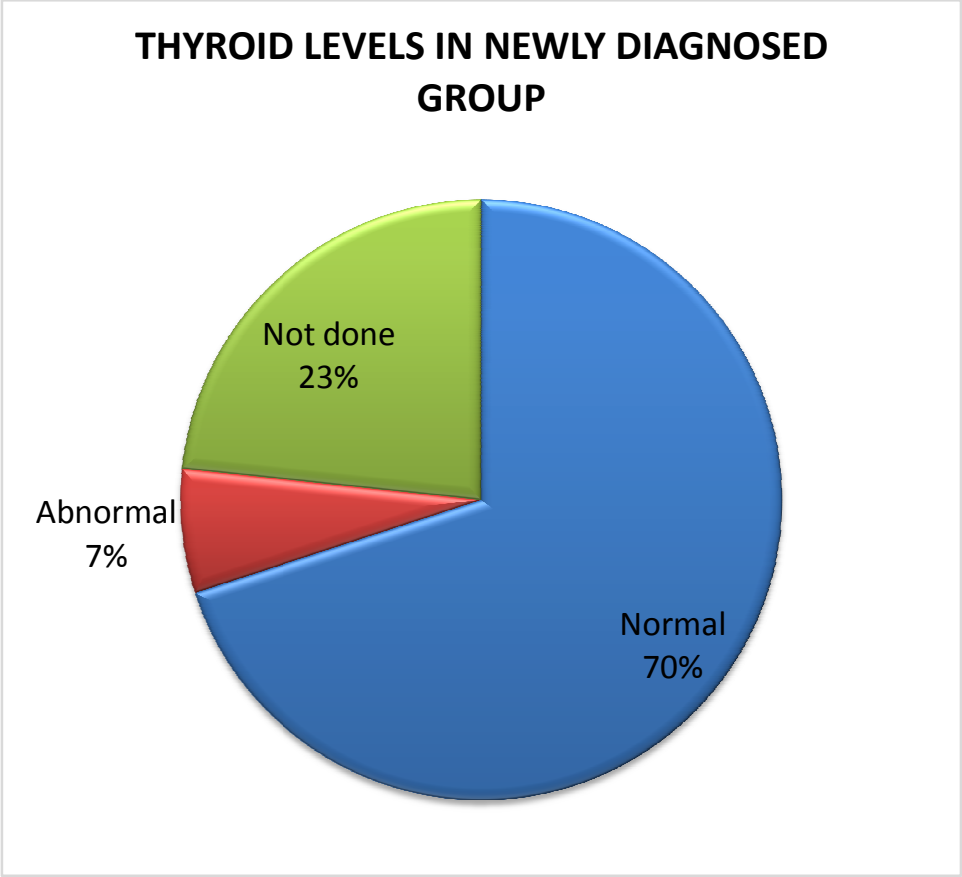
66.67% of newly diagnosed and 60% of already diagnosed patients have attended outpatient department for menstrual abnormality either for oligomenorrhea or amenorrhea. Rest of the patients came for infertility treatment.

Figure 4 represents the marital status of the study population



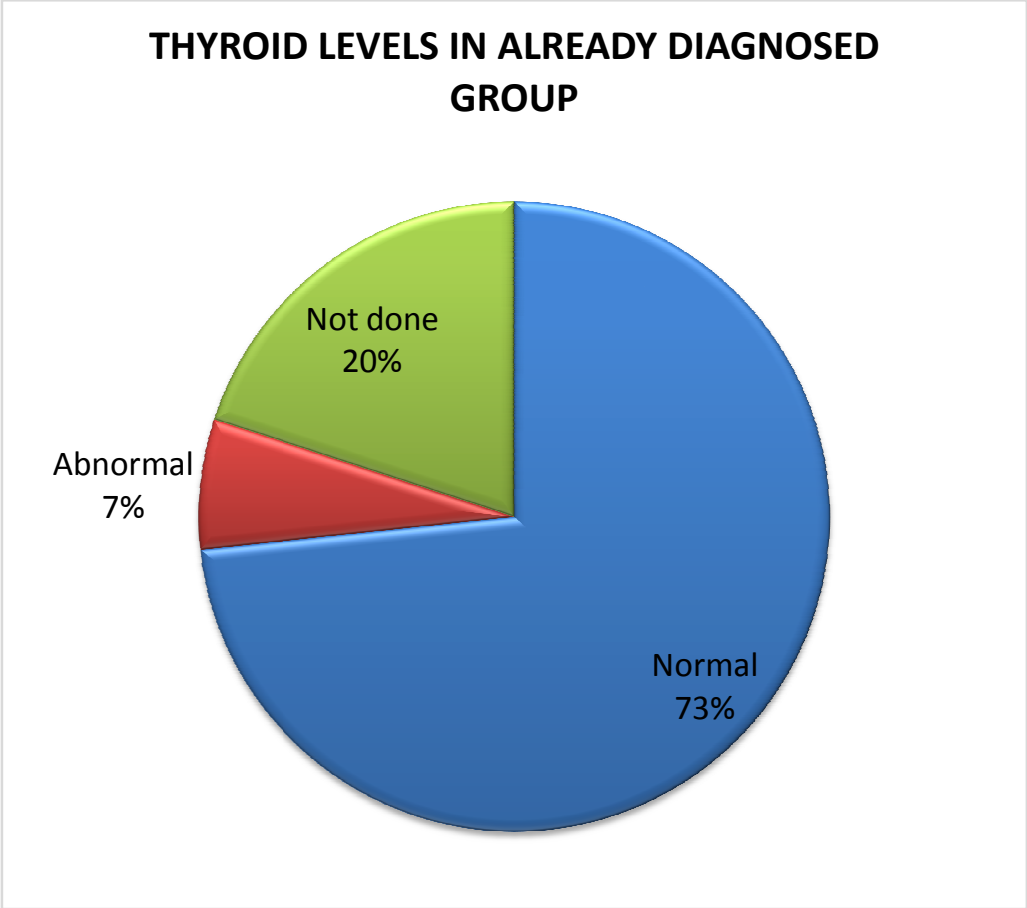
60% of the newly diagnosed patients and 80% of the already diagnosed patients were unmarried.40% of the newly diagnosed patients and 20% of the already diagnosed patients were married.

Figure 5 represents the newly diagnosed study population with thyroid disease



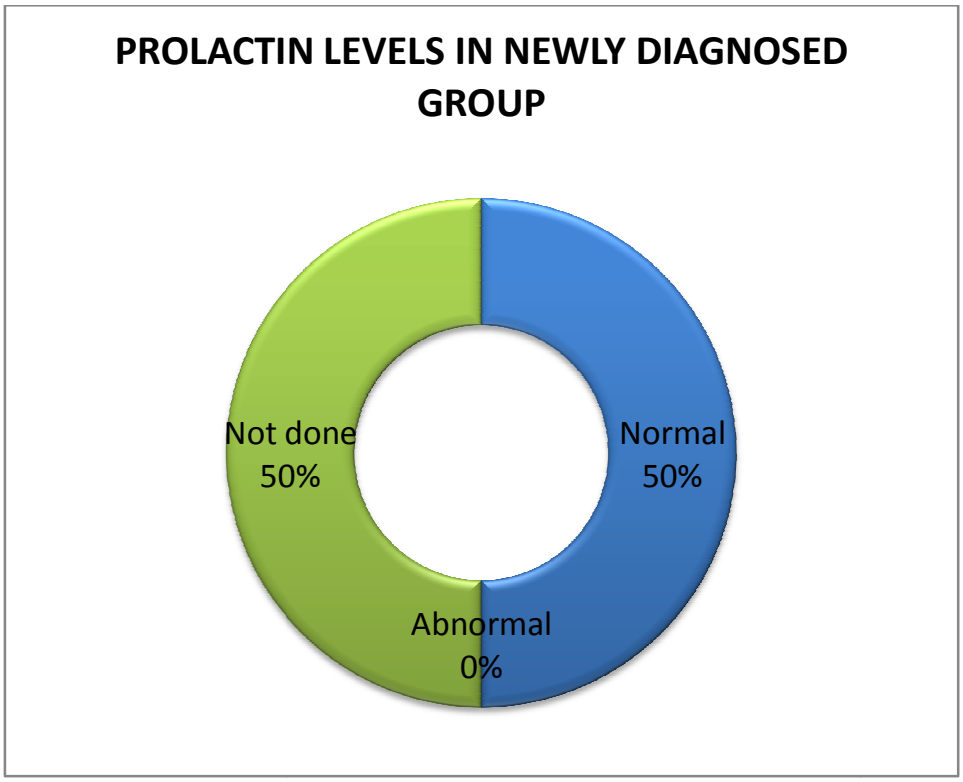
6.67% of the newly diagnosed population have thyroid.

Figure 6 represents the already diagnosed study population with thyroid



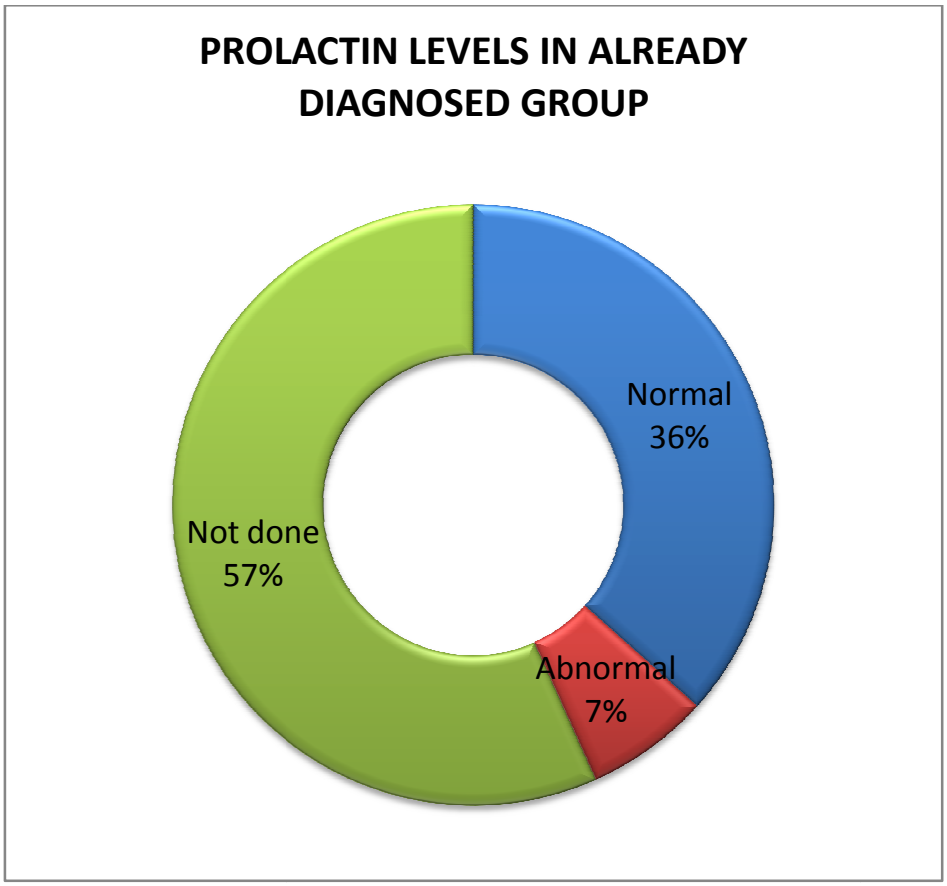
6.67% of the already diagnosed population have thyroid.

Figure 7 represents the newly diagnosed study population with prolactin levels



50% of the newly diagnosed study population had normal prolactin levels.

Figure 8 represents the already diagnosed PCOS study population with Prolactin levels



36% of the study population had normal prolactin levels.

Figure 9 represents the therapies in the already diagnosed group

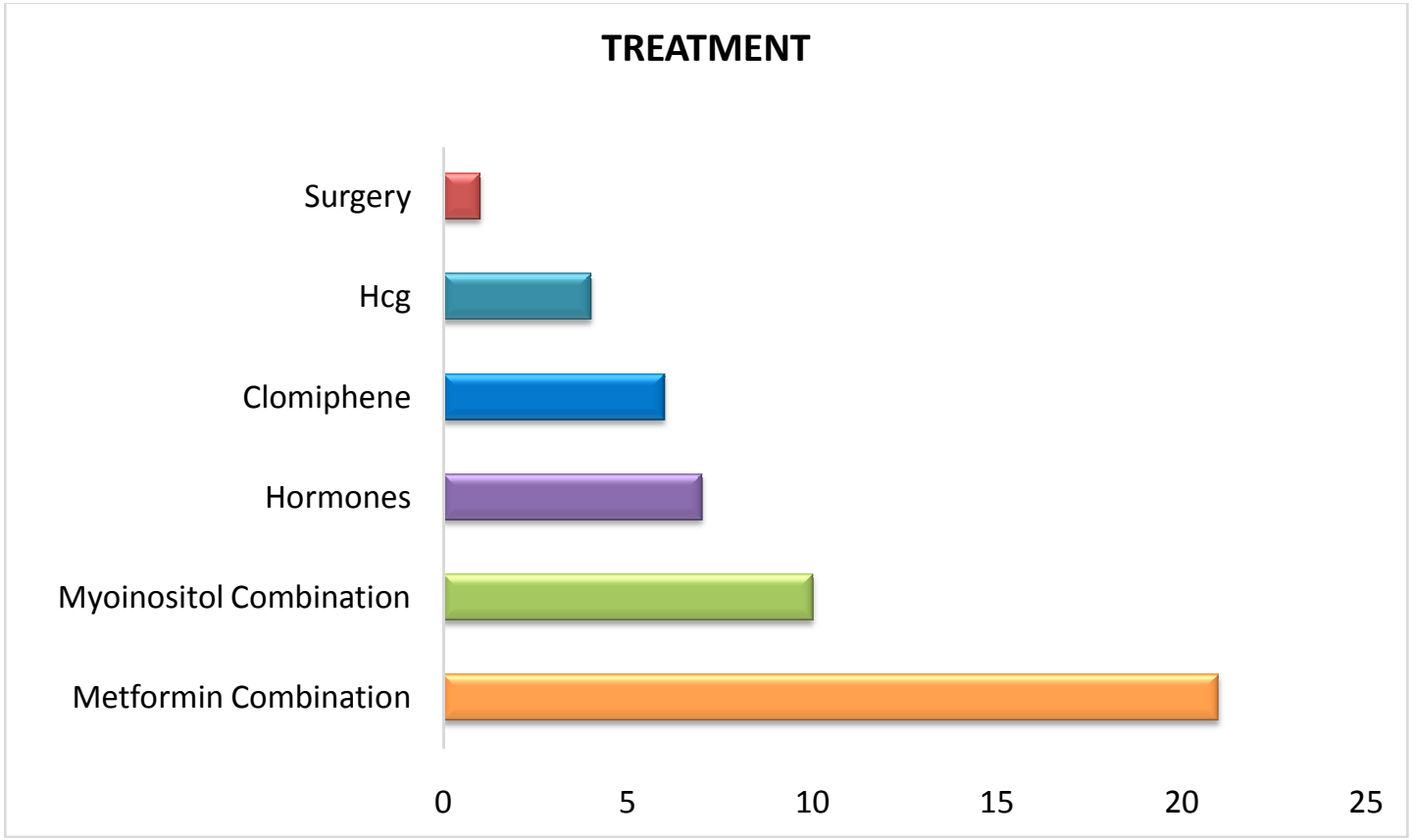
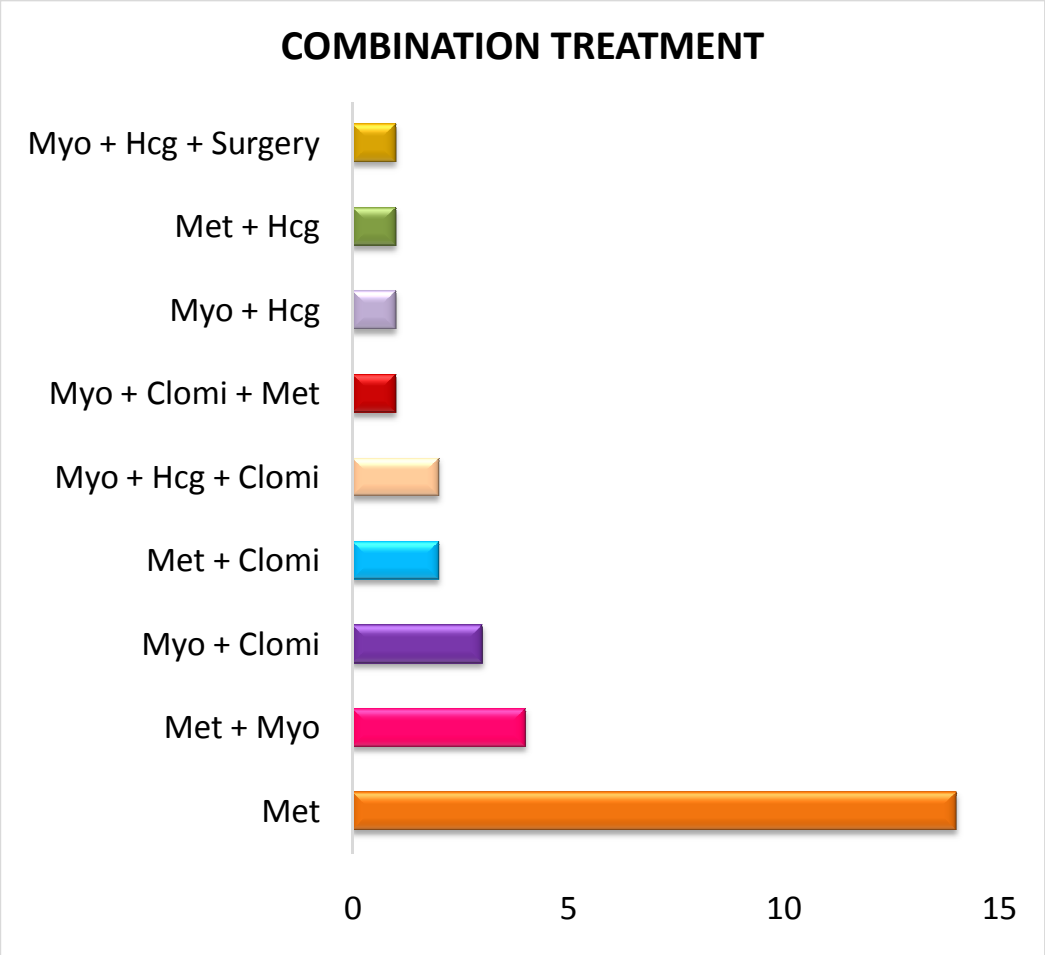


Table 1 represents the percentage of drug treatment in the same group

Drug	Percentage
Metformin Combination	70
Myoinositol Combination	33.33
Hormones	23.33
Clomiphene citrate	20
Human Chorionic Gonodotropin injection	13.33
Laproscopic Ovarian Drilling	3.3

70% of the study population in already diagnosed group were on Metformin combination and 33.33% of the study population were in Myoinositol Combination.

Figure 10 represents the combination of drugs



Met – Metformin

Myo – Myo-inositol

Clomi – Clomiphene citrate

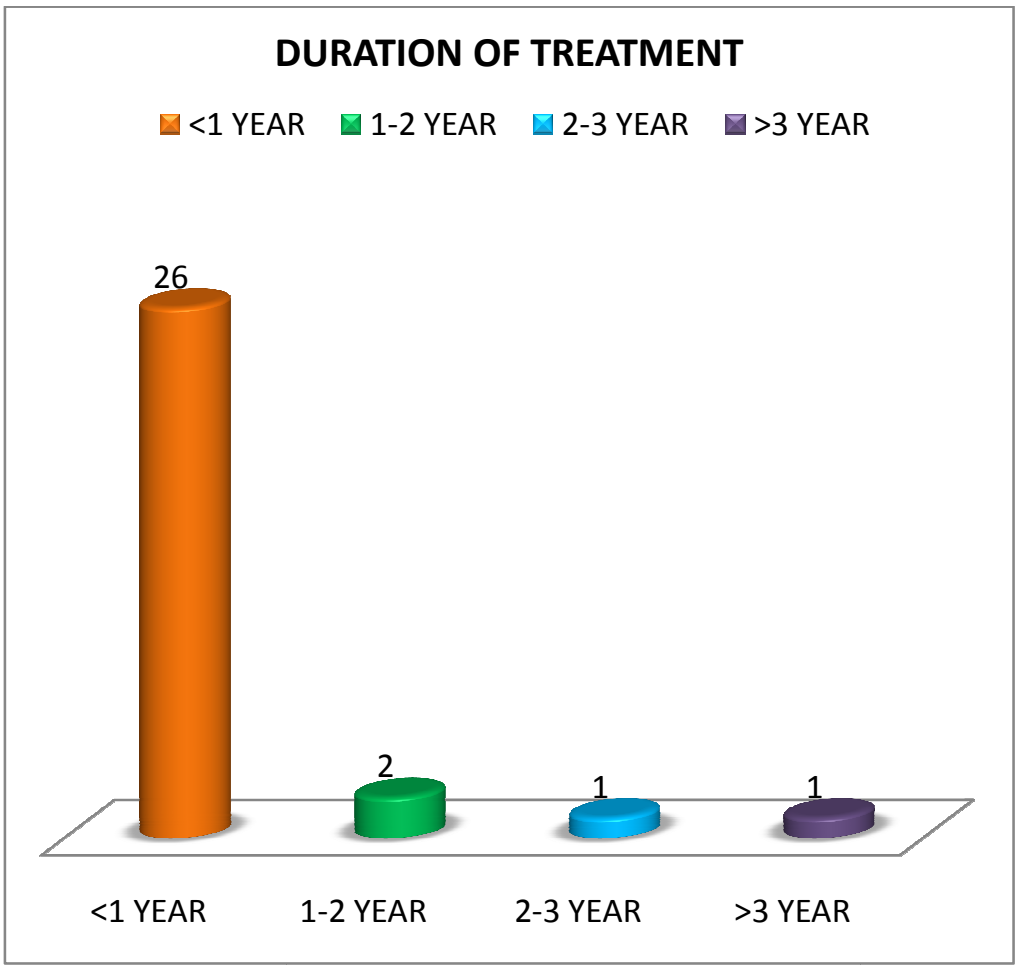
Hcg – Human Chorionic Gonadotropin

Table 2 represents the combinations of drug

Drugs	Percentage
Only Metformin	46.67
Metformin + Myoinositol	13.33
Myoinositol + Clomiphene citrate	10
Metformin+ Clomiphene citrate	6.67
Myoinositol + HCG + Clomiphene citrate	6.67
Myoinositol + Metformin + Clomiphene citrate	6.67
Myoinositol + HCG	3.33
Metformin + HCG	3.33
Myoinositol + HCG + LOD	3.33

46.67% of patients were prescribed with Metformin alone and metformin along with myoinositol were prescribed for 13.33% of patients. Myoinositol with Clomiphene citrate were given for 10% of patients and Metformin and Clomiphene citrate were given for 6.67% of patients.

Figure 11 represents the duration of treatment



26 patients (86.67%) were on treatment for less than 1 year.

Table 3 and Table 4 represents the comparison of hs-CRP for the three groups.

Table 3: One way ANOVA:

	F	p-value
Between groups Within Groups	0.025	0.975

Table 4: Post Hoc (LSD) analysis:

Between Groups Comparison	Mean Difference	p-value
I and II	0.00667	0.963
I and III	0.02433	0.867
II and III	0.03100	0.831

There was no difference between the newly diagnosed and already diagnosed study population and there was no statistically significant decrease in the hs-CRP level in between the groups.

Figure 12 shows the hs-CRP levels for the three groups

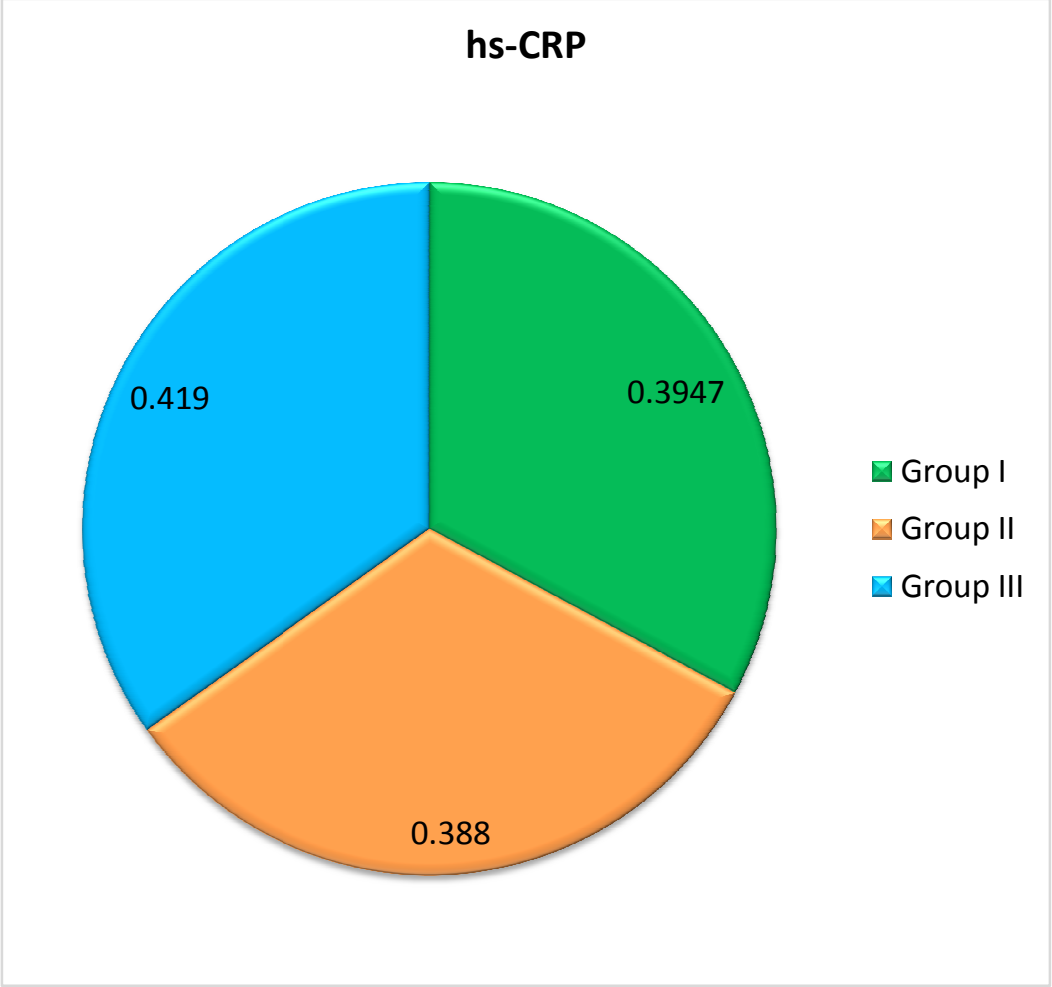


Table 4 and Table 5 represents the IL-6 level. There was no difference between the newly diagnosed and already diagnosed study population and there was no statistically significant decrease in the IL-6 level.

Table 4: One-way ANOVA:

	F	p-value
Between groups Within groups	0.293	0.746

Table 5: Post hoc (LSD) analysis:

Between Groups Comparison	Mean Difference	P-value
I and II	56.23703	0.746
I and III	57.06563	0.506
II and III	0.82860	0.992

Figure 13 represents the IL-6 levels between three groups

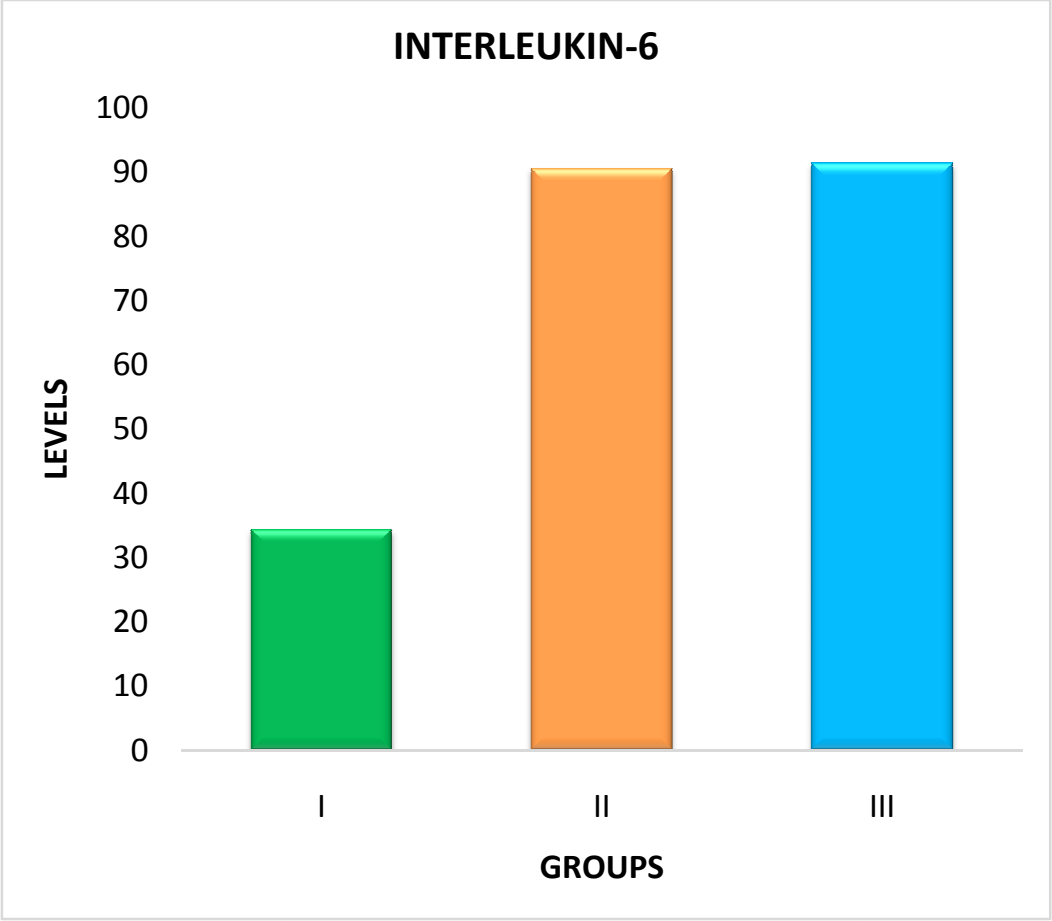


Table 6 and Table 7 represents the IL-18 levels. We were not able to establish a impact between the two population.

Table 6: One way ANOVA:

	F	p-value
Between groups Within Groups	1.954	0.148

Table 7: Post hoc (LSD) analysis:

Between Groups Comparison	Mean Difference	P-value
I and II	133.417	0.114
I and III	150.75323	0.074
II and III	17.33650	0.836

Figure 14 shows the IL-18 levels between three groups

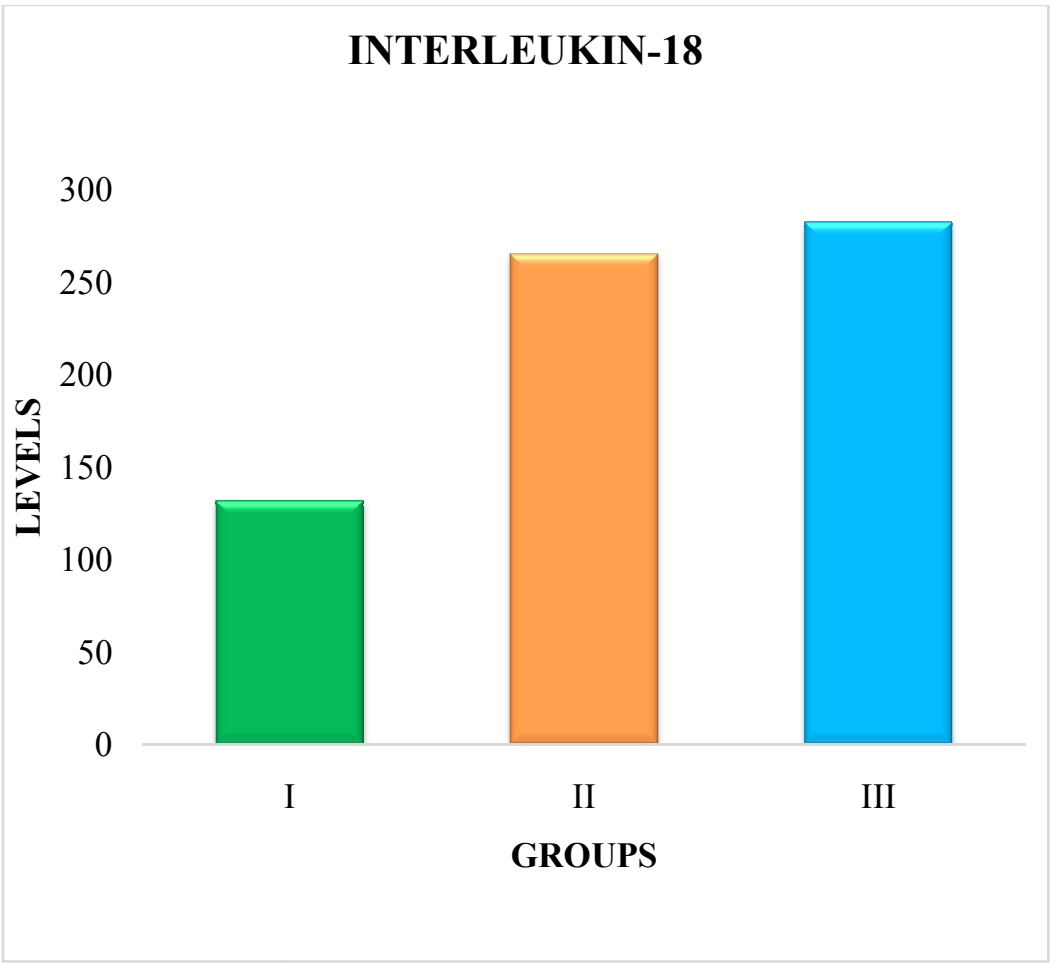


Figure 15 represents the hs-CRP levels with regard to the duration of treatment. When mean values were compared between the patients whose duration of treatment was less than six months and more than six months the values has reduced but it was not statistically significant (p=0.188).

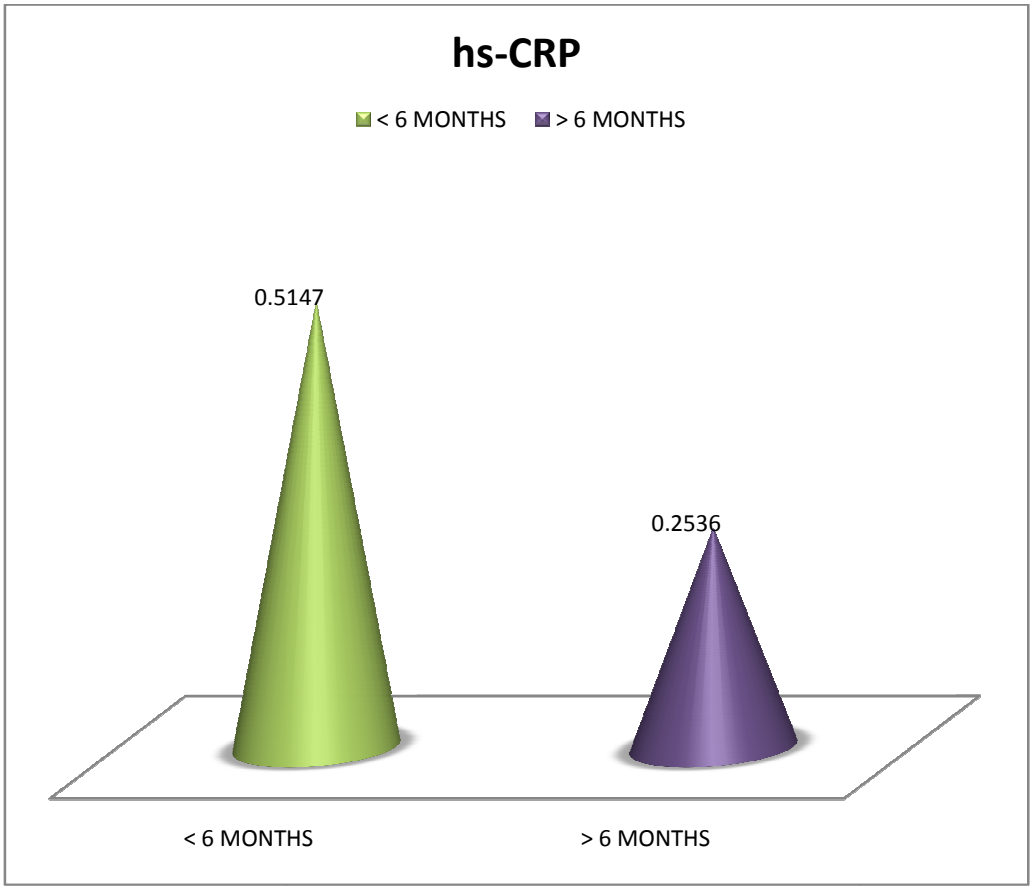


Figure 16 represents the IL-6 levels with respect to the duration of treatment. There was no statistical significance (p=0.230) between the patients with less than six months duration of treatment and more than six months of treatment but their mean values have reduced.

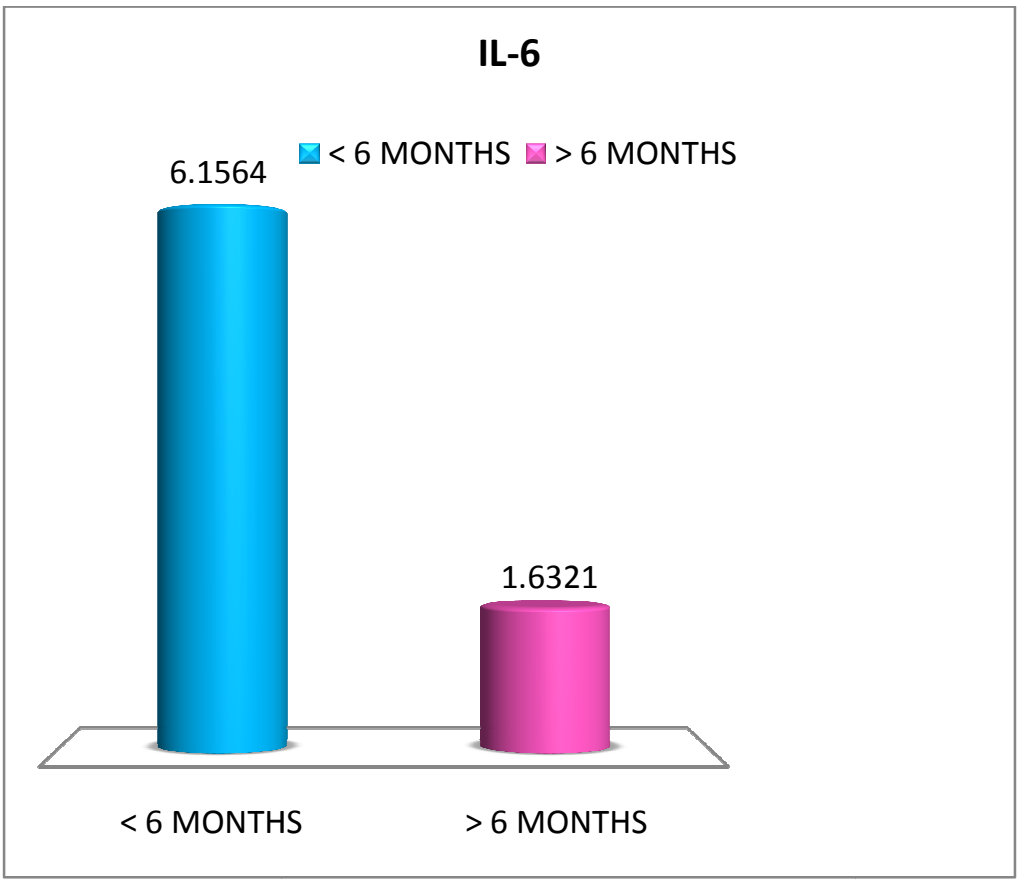


Figure 17 represents the IL-18 levels with regard to the duration of treatment. There was a reduction in the mean values between the less than six months and more than six months treatment but there was no statistical significance between them (p=0.441).

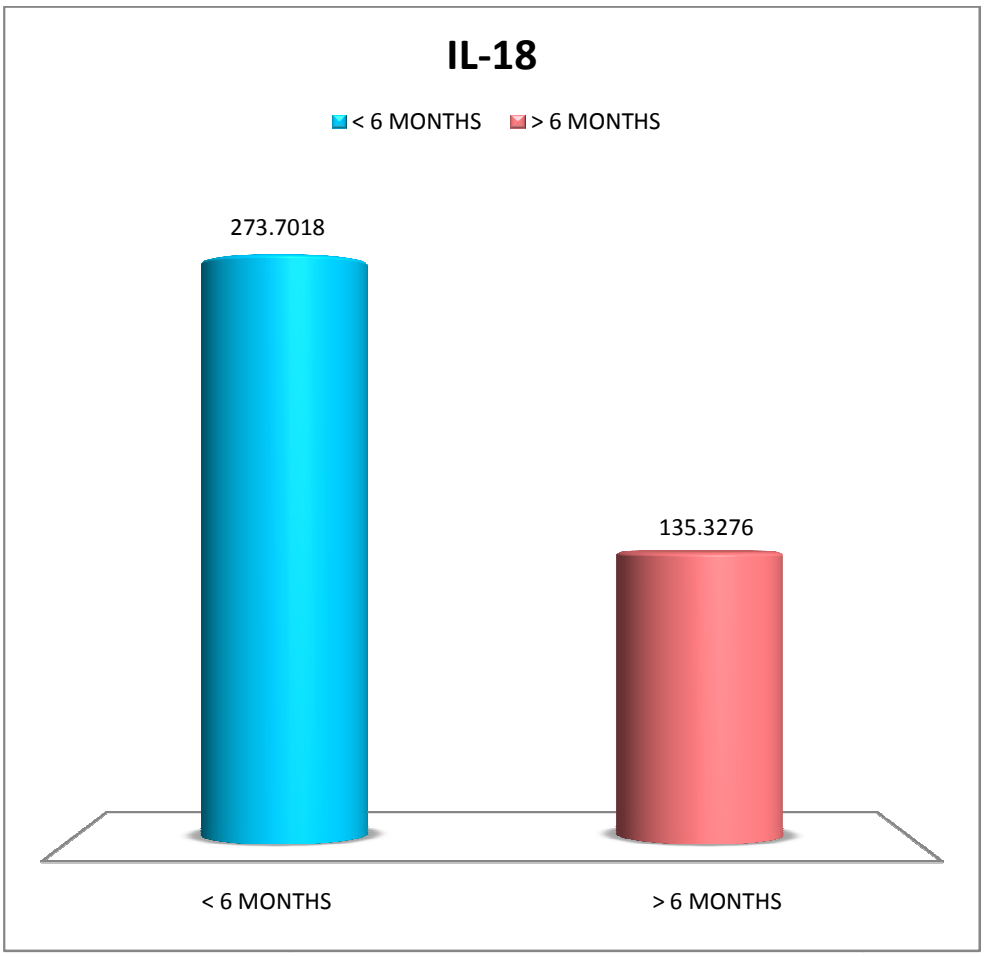


Figure 18 represents the IL-6 levels

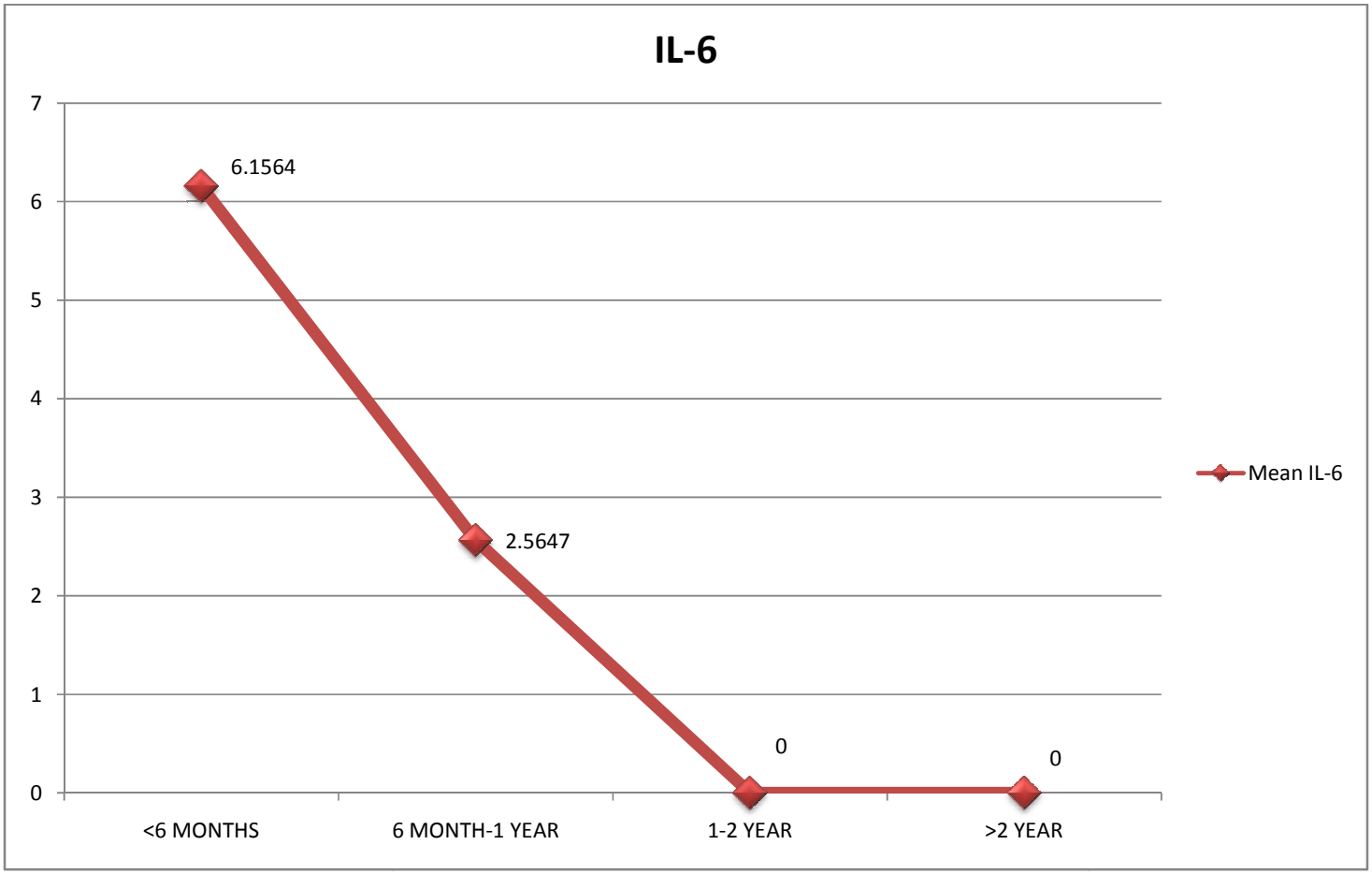


Figure 19 represents the IL-18 levels

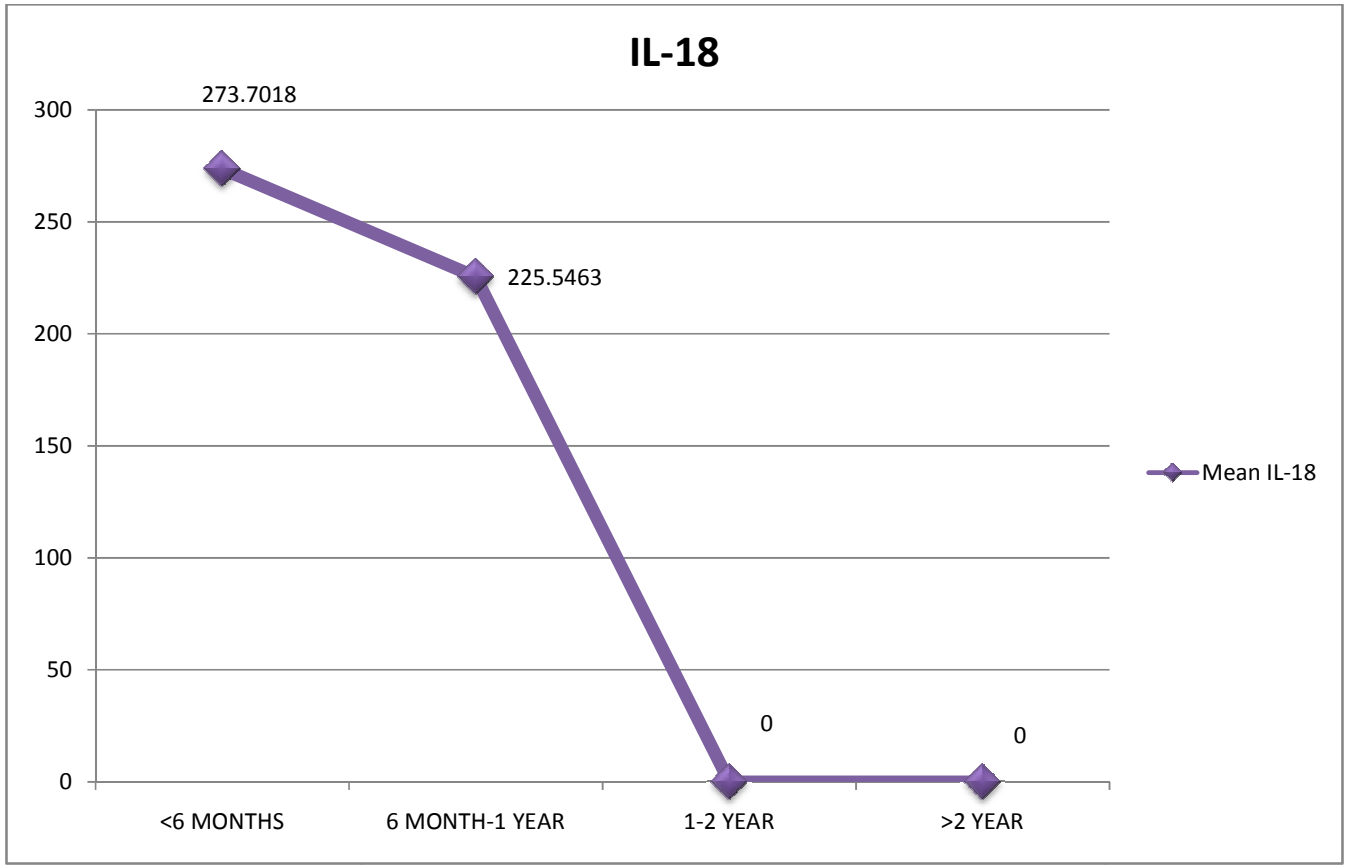
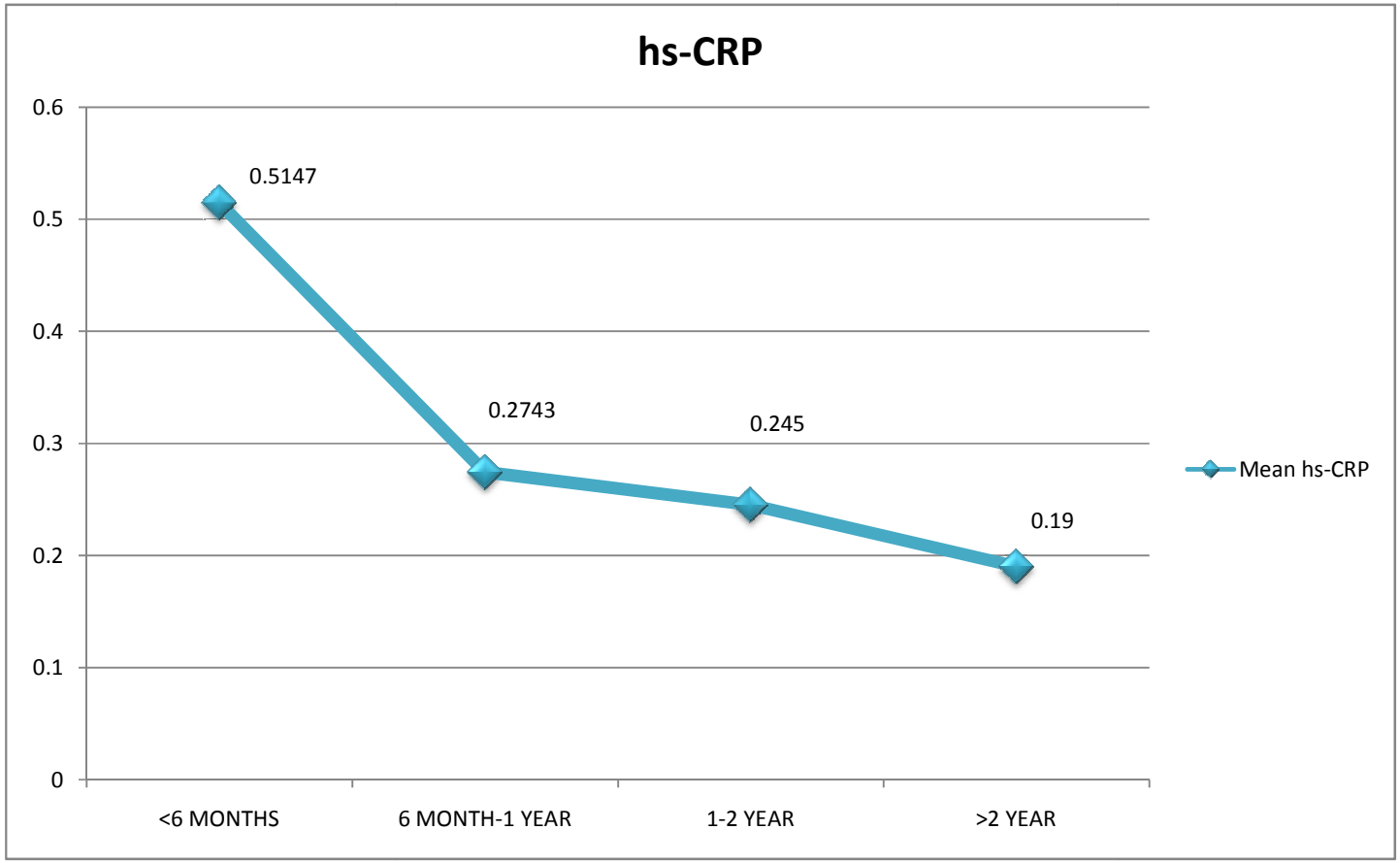


Figure 20 represents the hs-CRP levels



DISCUSSION

This study was intended to evaluate the status of inflammation, by measuring hs-CRP, IL-6 and IL-18, in PCOS patients in Indian population. It was also aimed to assess the influence of drugs prescribed in PCOS patients on the inflammation.

Polycystic Ovary Syndrome is an endocrine disorder which is very common in the female population in the reproductive age group[24]. Most women shows the features of metabolic syndrome. It is also concomitant with hypertension, diabetes, dyslipidaemia, gestational type 2 diabetes[154].

PCOS is associated with obesity and also increased incidence of insulin resistance[155]. There is also increased risk of cardiovascular complications. The inflammation which is of chronic low grade is related to PCOS. The C-reactive protein levels is an acute phase reactant and is circulating marker of cardiovascular events. CRP > 3mg/L in a patient is equal for metabolic syndrome as in ATP III guidelines. IL-6, TNF-α, IL-18 concentrations in serum are the predictors of cardiovascular events[3]. Hence, in this study we proposed to measure the IL-6, IL-18 and hs-CRP levels in PCOS patients in Indian population. There are various studies showing the significance of IL-6, IL-18 and

hs-CRP in PCOS in other countries. There are no studies showing this significance in Indian Population.

There were many therapies of polycystic ovarian syndrome include clomiphene citrate, insulin sensitizing agents, gonadotropins and gonadotropin- releasing hormone (GnRH) analogs, laparoscopic ovarian drilling and the application of assisted reproduction techniques. Apart from this weight loss, diet and exercise have been shown to improve hyperinsulinemia, menstrual abnormalities and ovulation rates[156]. In previous studies drugs like metformin and thioazolidinones like pioglitazone, rosiglitazone decreases insulin resistance and also known to decrease the inflammation. Other drugs like myo-inositol, clomiphene citrate improves ovulation alone. Their role in inflammation was not known in PCOS.

Ninety participants were recruited in this study. These participants were divided into three groups as normal controls, newly diagnosed patients and already diagnosed on treatment patients. The age group in normal controls was 40% in 3rd decade and 60% in 4th decade. The age group in newly diagnosed was 73.3% and already diagnosed was 66.67% in the 3rd decade whereas in 4th decade it was 13.33% and 26.67% respectively. In normal controls there were no patients in <20 years of

age whereas in newly diagnosed and already diagnosed on treatment groups 13.33% and 6.67% were under 20 years of age respectively and no patients in the age of more than 40 in all the three groups (Figure 1).

On comparing patients' weight, there were 53.33% in normal controls, 43.33% in newly diagnosed and 50% in already on treatment groups have Normal weight. Overweight was 30%, 36.67%, 33.33% in Normal controls, newly diagnosed, already on treatment groups respectively. 13.33%, 3.33%, 13.33% participants were obese in normal controls, newly diagnosed, already on treatment groups. Only 3.33% were underweight in both normal and already diagnosed on treatment group participants whereas in newly diagnosed there were 16.67% (Figure 2).

In newly diagnosed and already diagnosed on treatment group, participants attended outpatient department of Obstetrics and Gynaecology for both menstrual abnormalities either amenorrhoea or oligomenorrhoea and Infertility. Out of thirty participants in newly diagnosed PCOS, 66.67% and 33.33% attended the outpatient for menstrual abnormalities and for infertility. In already diagnosed patients 60% and 40% attended due to menstrual abnormalities and infertility. Thus participants in both the groups attended the outpatient department mostly for oligo/amenorrhoea (Figure 3). In newly diagnosed patients

60% of the patients were married and in already diagnosed patients 80% of the patients were married. In married patients most of the patients came with the history of infertility (Figure 4).

Only 6.67% in newly diagnosed and 6.67% in already diagnosed patients were hypothyroid and they were on treatment on levothyroxine. Seven patients (23.33%) in newly diagnosed and six patients (20%) in already diagnosed patients' level of thyroid was not found. Remaining patients were normal in both the groups (70% and 73%) (Fig.5 &6).

In case of prolactin levels, in newly diagnosed fifteen patients (50%) have normal levels and for another fifteen patients (50%) levels were not found. In already diagnosed on treatment group it was normal for eleven patients (36%) and abnormal for 2 patients (6.67%). It was not checked for seventeen patients (56.6%) (Fig.7&8).

Low grade chronic inflammation is associated with Polycystic Ovary Syndrome and it can be assessed using inflammatory markers like IL-6, IL-18 and hs-CRP. These are strong predictors of cardiovascular complications. An article proposed by Samy et al compared PCOS patients with BMI matched controls and reported that there is a significant increase in hs-CRP, IL-6 and TNF-α only in the obese group of patients. They have also showed a positive correlation between these

inflammatory markers and HOMA index which was used to measure the insulin resistance[157]. Kelly et al. reported a study exhibiting an increase in CRP which shows a low grade chronic inflammation in PCOS patients. In this study they showed mean value of PCOS patients was greater than normal controls[3].

Here in this study there was no significant increase in the CRP levels (p=0.975). There was no significant difference between the newly diagnosed and already diagnosed patients suggesting that hs-CRP didn't rise significantly in already diagnosed group as they were on treatment (Table 3 & 4) (Figure 13).

In the same way there was also no significant changes in the IL-6 levels (p=0.746). There was an increase in their mean values when compared to normal controls. Mean value for normal controls was 34.35$\pm$93.88 whereas for newly diagnosed patients it was 90.58$\pm$308.62 and for already diagnosed it was 91.41$\pm$476.6 suggesting there was a rise in IL-6 levels in PCOS when compared to normal controls but that was not significant. Between newly diagnosed patients and already diagnosed patients there was no much difference between the levels (Table 5 &6) (Figure 14).

Another study by Hector et al showed there was an increase in IL-18 independent of obesity[158]. Here in this study the results showed there was an increase in the mean values of IL-18 in PCOS patients when compared to normal controls. The mean value for Normal control was 131.4±224.17 whereas for newly diagnosed and already diagnosed group of patients it was 264.86±316.33 and 282.2±403.92 respectively. The difference was not statistically significant (p=0.148) (Table 7&8) (Figure 14). There was also, as in IL-6, no difference in newly diagnosed and already diagnosed patients.

When we considered the treatment prescribed to the patients in already diagnosed group, most of the patients were in Metformin combination (Table 1). Nearly 70% of patients were in metformin combination (Figure 9). Metformin was given along with myoinositol, clomiphene citrate, Human Chorionic Gonadotropins (HCG). Only 46.67% patients were on monotherapy of metformin (Table 2).

The other common drug prescribed was myoinositol combination. It was around 33.33% patients who were prescribed with this combination. It was also given along with metformin, clomiphene citrate and HCG. Metformin along with myoinositol was given to 13.3% of patients.

Menstrual abnormality was one of the chief complaints of the patient (60%) in this group. So they were given low dose of medroxy progesterone, ethinylestradiol, Cyproterone acetate for about seven patients (23.3%). As patients come for infertility (40%) they were given clomiphene citrate (20%) and HCG (13.3%) injection. Laparoscopic Ovarian Drilling was done for one patient out of the thirty patient and she was prescribed with myoinositol and HCG injection later.

86.67% of patients were on these treatment for less than 1 year and only 13.33% of patients were on these therapies for more than 1 year (Figure 11).

Mekkawi et al stated that there was a statistical significant change in the Interleukin-6 and Interleukin-18 levels before and after metformin therapy showing metformin decreases the inflammatory markers in PCOS patients[77]. In previous studies it was known that there was an increase in the Nuclear Factor κ B (NFκB) in PCOS. Metformin inhibits NFκB which inhibits IL-1β and inhibits the production of pro inflammatory gene[75].

In this study, the mean $\pm$ SD for hs-CRP, IL-6,IL-18 were decreased between the patients who were on treatment for less than six months and more than six months (Figure 15,16,17). But there was no statistical

significance in the hs-CRP (p=0.188), IL-6 (p=0.230) and IL-18 (p=0.441) between these two group of patients. We could also see a decrease in the mean values of hs-CRP, IL-6 and IL-18 as there was an increase in the duration of treatment (Figure 18, 19, 20).

Our results were consistent with Mohlig et al. who showed there was no substantial decrease in CRP and IL-6 levels. In their study these levels were increased in both normal and PCOS obese patients compared to the lean patients. There was a positive relationship between the obesity, insulin resistance and inflammatory markers than the PCOS itself. When a linear regression was done in their study it showed obesity was the one which increases the inflammatory marker than the insulin resistance[52]. Another study by Escobar-Morreale et al. also showed obesity causes an increase in the inflammatory marker and not the disease itself[159].

In this study we had only 13.3% of obese study population in the already diagnosed and on treatment group and 3.33% in the newly diagnosed PCOS group. Also in this study there were about 86.7% of already diagnosed group of patients' on treatment for less than one year. So even though there was a fall in the mean values of inflammatory markers there was no statistical significance in them.

The lack of statistical significance may be due to small sample size in each group and wide range of values between the patients. The duration of treatment which was less than one year in most of the patients and hence the effect of drugs on the inflammatory markers could not be assessed. As this was a cross sectional study and blood samples were taken at one time the difference in the inflammatory markers could not be studied for the same group of patients. The influence of drugs prescribed were also in combination for most of the patients and so they could not be studied individually.

SUMMARY & CONCLUSIONS:

Though there are various pharmacological drugs available to treat PCOS, only few drugs targeted in reduction of inflammation. There were various studies, as we quoted earlier, demonstrating the effect of inflammation on PCOS. These studies were all done in other parts of the world. There were very less number of studies done in India. So we proposed to study the status of inflammation on PCOS in Indian population in particular South Indian population.

The pro inflammatory cytokines were produced when NFκB was stimulated. Metformin inhibits this NFκB and thus reduces the inflammation. Other drugs used in PCOS in practise like myoinositol, clomiphene citrate, hormones target at the disease per se and not on the inflammation produced by the disease.

This study has shown that mean values of inflammatory markers were increased in Polycystic Ovary Syndrome (PCOS) in newly diagnosed and already on treatment for PCOS. 86.7% of the patients who were on already treatment for PCOS group were on treatment for less than one year. It has shown that there was an inversely proportional relationship between the mean values of inflammatory markers and duration of treatment.

We suggest larger follow up studies with longer duration of treatment so that influence of drugs on the inflammatory markers can be studied exhaustively in Indian population. Further studies are needed to demonstrate the effects of drugs for PCOS targeting on the inflammatory pathway so that these drugs not only brings down the severity of PCOS but also reduces the cardiovascular risk factors associated with it.

BIBLIOGRAPHY

1. Walker JR. Mortalilty,Morbidity and health. Population Economics. Spring 2012.

2. Nidhi R Padmalatha V Nagarathna R Amritanshu R. Prevalence of polycystic ovarian syndrome in Indian adolescents. Journal of pediatric and adolescent gynecology. 2011; 24(4):223-7.

3. Chris C.J.Kelly, Helen Lyall, John R.Petrie et al. Low grade chronic Inflammation in Women with Polycystic Ovarian Syndrome. The Journal of Clinical Endocrinology & Metabolism.2001,2453-2455

4. Polson DW, Adams J, Wadsworth J, et al. Polycystic Ovaries- a common finding in normal women. Lancet 1988; 1:870-872.

5. Stein IF, Leventhal ML. Amennorhea associated with bilateral polycystic ovaries. Am J ObstetGynecol 1935; 29: 181-191.

6. Takahashi K, Eda Y, Abu Musa A, et al. Transvaginal ultrasound imaging, histopathology and endocrinopathy in patients with polycystic ovarian syndrome. Hum Reprod 1994; 9:1231-1236.

7. Campbell S,Goessens L, Goswamy R, et al. Real-time ultrasonography for determination of ovarian morphology and volume. Lancet 1982; 1:425-428.

8. Orsini LF, Rizzo N, Calderoni P, et al. Ultrasound monitoring of ovarian follicular development: a comparision of real-time and static scanning techniques. J Clin Ultrasound 1983; 11:207-211.

9. Orsini LF, Venturoli S, Lorusso R, et al. Ultrasonic findings in polycystic ovarian disease. FertilSteril 1985; 43: 709-714.

10. Adams J, Polson DW, Franks S. Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsuitism. Br Med J 1986;293:355-359.

11. Adams J, Polson DW, Abdulwahid N, et al. Multifollicular ovaries: clinical and endocrine features and response to pulsatile gonadotrophin releasing hormone. Lancet 1985; 2:1375-1379.

12. Balen AH, Conway GS, Kaltsas G, et al. Polycystic ovary syndrome: the spectrum of the disorder in 1741 patients. Hum Reprod 1995;10:2107-2111.

13. Conway GS, Honour JW, Jacobs HS. Heterogeneity of the polycystic ovary syndrome; clinical, endocrine and ultrasound features in 556 patients. ClinEndocrinol (Oxf) 1989; 30:459-470.

14. Kiddy DS, Sharp PS, White DM, et al. Differences in clinical and endocrine features between obese and non-obese subjects with polycystic ovary syndrome: an analysis of 256 consecutive cases. ClinEndocrinol (Oxf) 1990; 32:213-220.

15. Fox R, Corrigan E, Thomas PA, et al. The diagnosis of polycystic ovaries in women with oligo-amennorhea: predictive power of endocrine tests. ClinEndocrinol (Oxf) 1991; 34:127-131.

16. Abdel Gadir A, Khatim MS, Mowafi RS, et al. Implication of ultrasonically diagnosed polycystic ovaries, Correlations with basal hormonal profiles. Hum Reprod 1992; 7: 453-457.

17. Clayton RN, Ogden V, Hodgkinson J, et al. How common are polycystic ovaries in normal women and what is their significance for the fertility of the population? ClinEndocrinol (Oxf) 1992; 37: 127-134.

18. Farquhar Cm, Birdsall M, Manning P, et al. The prevalence of polycystic ovaries on ultrasound scanning in a population of randomly selected women. Aust NZ J ObstetGynaecol 1994; 34:67-72.

19. Saxton DW, Farquhar CM, Rae T, et al. Accuracy of ultrasound measurements of female pelvic organs. Br J ObstetGynaecol 1990; 97: 695-699.

20. Takahashi K, Eda Y, Abu Musa A, et al. Transvaginal ultrasound imaging, histopathology and endocrinopathy in patients with polycystic ovarian syndrome. Hum Reprod1994 ;9:1231-1236.

21. Balen AH, Conway GS, Kaltsas G, et al. Polycystic ovary syndrome: the spectrum of the disorder in 1741 patients. Human Reprod 1995; 10:2107-2111.

22. Franks S. Polycystic ovary syndrome: a changing perspective. ClinEndocrinol (Oxf) 1989; 31:87-120.

23. Conway GS, Honour JW, Jacobs HS. Heterogeneity of the polycystic ovary syndrome: clinical, endocrine and ultrasound features in 556 patients. ClinEndocrinol (Oxf) 1989; 30:459-470.

24. Knochenhauer ES, Key TJ, Kahsar-Miller M, et al. Prevalence of the polycystic ovary syndrome in unselected Black and White Women of the Southeastern United States: A prospective study. J ClinEndocrinolMetab 1998;83:3078-3082.

25. Wijeyaratne CN, Balen AH, Barth J, et al. Clinical Manifestation and insulin resistance(IR) in polycystic ovary syndrome among South Asians and Caucacians: is there a difference? ClinEndocrinol (Oxf)2002; 57:343-350.

26. Rosenfield RL, Barnes RB, Cara JB, et al. Dysregulation of cytochrome P450c17α as the cause of polycystic ovarian syndrome. FertilSteril 1990; 53:785-791.

27. White DW, Leigh A, Wilson C et al. Gonadotrophin and gonadal steroid response to a single dose of a long-acting agonist of gonadotrophin-releasing hormone in ovulatory and anovulatory women with polycystic ovary syndrome. ClinEndocrinol (Oxf) 1995; 42:475-481.

28. Franks S, White D, Gilling-Smith C, Carey A et al. Hypersecretion of androgens by polycystic ovaries: the role of genetic factors in the regulation of cytochrome P450c17α. Balliere'sClinEndocrinolMetab 1996; 10:193-203.

29. Webber LJ, Stubbs S, Stark J et al. Formation and early development of follicles in the polycystic ovary. Lancet 2003; 362: 1017-1021.

30. Fauser BCJM, Pache TD, Lamberts WJ et al. Serum bioactive and immunoactiveleutenising hormone and follicle stimulating hormone levels in women with cycle abnormalities, with or without polycystic ovary disease. J ClinEndocrinolMetab 1991; 73:811-817.

31. Taylor AE, McCourt B, Martin K et al. Determinants of abnormal gonadotrophin secretion in clinically defined women with PCOS. J ClinEndocrinolMetab 1998; 66: 165-172.

32. Waldstreicher J, Santoro NF, Hall HJE et al. Hyperfunction of the hypothalamic-pituitary axis in women with polycystic ovarian

disease: Indirect evidence of partial gonadotroph desensitization. J ClinEndocrinolMetab 1998; 66:165-172.

33. Balen AH, Conway GS, Kaltsas G et al. Polycystic ovary syndrome. The spectrum of the disorder in 1741 patients. Hum Reprod 1995; 10:2705-2712.

34. Laven JS, Imani B, Eijkemans MJ et al. New approaches to PCOS and other forms of anovulation. ObstetGynecolSurv 2002; 57:755-767.

35. Van Santbrink EJ, Hop WC, Fauser BC. Classification of normogonadotropin infertility: Polycystic ovaries diagnosed ny ultrasound versus endocrine charecteristicsoc PCOS. FertilSteril 1997; 67:452-458.

36. Balen AH, Tan SL Jacobs HS. Hypersecretion of leutenising hormone-a significant cause of subfertility and miscarriage. Br J ObstetGynaecol 1993; 100:1082-1089.

37. Schally AV, Arimura A, Kastin AJ et al. Gonadotropin-releasing hormone: one polypeptide regulates secretion of leutenising andfollicle stimulating hormones. Science 1971; 173: 1036-1037.

38. Dalkin AC, Haisenleder DJ, Ortolano GA et al. The frequency of GnRH stimulation differentially regulates gonadotropin subunit mRNA expression. Endocrinol 1989; 125:917-924.

39. Bridges NA, Cooke A, Healy MJR et al. Standards for ovarian volume in childhood and puberty. FertilSteril 1993; 60:456-460.

40. Tartaglia LA, Dembski M, Weng X et al. Identification and expression cloning of a leptin receptor, Ob-R. Cell 1995; 83: 1-20.

41. Jones GL, Benes K, Clark TL et al. The polycystic ovary syndrome health -related quality of life questionnaire (PCOSQ): a validation. Hum Reprod 2004; 19:371-377.

42. ACE position statement on the Insulin Resistance Syndrome, EnocrPract. May/June 2003; Vol 9 (No 3).

43. Dunaif A, Xia J, Book C et al. 1995 Excessive Insulin receptor serine phosphorylation in cultured fibroblasts and in skeletal muscle: a potential mechanism for insulin resistance in the polycystic ovary syndrome. J Clin Invest 96:801-810.

44. Caro JF, Dohm LG, Pories WJ et al. 1989 cellular alteration in liver, skeletal muscle and adipose tissue responsible for insulin resistance in obesity and Type II diabetes. Diabetes Metab Rev 5:665-689.

45. Laure Morin-Papunen, Katrina Rautio, AimoRuokonen et al. Metformin Reduces Serum C-Reactive Protein Levels in Women with Polycystic Ovary Syndrome. J ClinEndocrinol Metab,2003; 88(10):4649-4654.

46. Carl de Luca, Jerrold M. Olefsky. Inflammation and insulin resistance. FEBS [Federation of European Biohemical Societies] Letters 582(2008) 97-105.

47. Escobar-Morreale HF, Batella. Carretero JI, Alvarez- Blasco F et al. The Polycystic Ovary syndrome associated with morbid obesity may resolve after weight loss induced by bariatric surgery. J ClinEndocrinolMetab. 2005; 90:6364-6369.

48. Escobar-MorrealeHF,Luque-Ramirez M, San Millan JL. The Molecular- genetic basis of functional hyperandrogenism and polycystic ovary syndrome. Endocr Rev.2005; 26:251-282.

49. Escobar-Morreale HF, Calvo RM, Sancho J et al. TNF-alpha and hyperandrogenism: a clinical, biochemical and molecular genetic study. J ClinEndocrinolMetab. 2001; 86:3761-3767.

50. Peral B, San Millan JL, Castello R et al. The methionine 196 arginine polymorphism in exon 6 of the TNF receptor 2 gene (TNFRSFIB) is associated with the polycystic ovary syndrome and hyperandrogenism.JClinEndocrinolMetab. 2002; 87:3977-3983.

51. Villuendas G, San Millan JL, Sancho J et al. The -597 G>A and -174 G>C polymorphism in the promoter of the IL-6 gene are associated with hyperandrogenism. J ClinEndocrinolMetab. 2002; 87:1134-1141.

52. Mohling M, Spranger J, Osterhoff M et al. The polycystic ovary syndrome per se is not associated with increased chronic inflammation. Eur J Endocrinol. 2004; 150:525-532.

53. Erdogan M, Karadeniz M, Berdeli A, et al. The relationship of interleukin-6- 174 G>C gene polymorphism with oxidative stress markers in Turkish polycystic ovary syndrome patients.JEndocrinol Invest. 2008; 31:624-629.

54. Escobar-Morreale HF, Calvo RM, Villuendas G, et al. Association of the polymorphism in the interleukin-6 receptor complex with obesity and hyperandrogenism. Obes Res. 2003; 11:987-996.

55. Jones TH. Interleukin-6 an endocrine cytokine. ClinEndocrinol (Oxf). 1994; 40:703-713.

56. Fried SK, Bunkin DA, Greenberg AS. Omental and subcutaneous adipose tissue of obese subjects release interleukin-6:depotdifference and regulation of glucocorticoid. J ClinEndocrinolMetab. 1998;83:847-850.

57. Purohit A, Ghilehik MW, Duncan L, et al. Aromatase activity and interleukin-6 production by normal and malignant breast tissues. J ClinEndocrinolMetab 1995; 80: 3052-3058.

58. Moshage HJ, Roelofs HM, van Pelt JF, et al. The effect of interleukin-1, interleukin-6 and its interrelationship on the

synthesis of serum amyloid A and C-reactive protein in primary cultures of adult human hepatocytes. BiochemBiophys Res Commun 1988; 155:112-117.

59. Ridker PM, Hennekens CH, Buring JE, et al. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med. 2000; 342:836-843.

60. Ouchi N, Kihara S, Funahashi T et al. Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. Circulation.2003; 107:671-674.

61. Okamura H, Tsutsui H, Kashiwamura S et al. Interkeukin-18: a novel cytokine that augments both innate and acquired immunity. AdvImmunol 70:281-312.

62. Antoni J Duleba, AnujaDokras, Is PCOS an inflammatory process. Fertil Steril2012; 97(1): 7-12.

63. Diamanti-Kandarakis E, Katsikis I, Piperi C et al. Increased serum advanced glycation end product is a distinct finding in lean women with polycystic ovary syndrome (PCOS). ClinEndocrinol (Oxf). 2008; 69:634-641.

64. Morin-Papunen LC, Duleba AJ, BloiguA et al. Chlamydia antibodies and self- reported symptoms of oligomennorhea and

hirsuitism: A new etiologic factor in polycystic ovary syndrome? Fertil Steril.2009.

65. Yavasoglu I, Kucuk M, Cildag B et al. A novel association between polycystic ovary syndrome and Helicobacter pylori. Am J Med Sci 2009;338:174-7.

66. Available from : http://www.news-medical.net/health/Metformin-History.aspx

67. Dronsfield A., Ellis P. Drug discovery: metformin and the control of diabetes. Education in chemistry November 2011; 185-87.

68. Klip A., LeiterL.A.Cellular Mechanism of Action of Metformin. Diabetes Care. 1990; 13:696-704.

69. Harborne LR, Sattar N, Norman JE, Fleming R. Metformin and weight loss in obese women with polycystic ovary syndrome: comparison of doses. J ClinEndocrinolMetab. 2005; 90(8):4593–4598.

70. Glueck CJ, Wang P, Goldenberg N, Sieve-Smith L. Pregnancy outcomes among women with polycystic ovary syndrome treated with metformin.HumReprod. 2002; 17(11):2858–2864.

71. Palomba S, Orio F Jr, Zullo F. What is the best first-step therapeutic approach in treating anovulatory infertility in patients

with polycystic ovary syndrome? Questions that are still unanswered. GynecolEndocrinol. 2007; 23(5):245–247.

72. Moll E, Bossuyt PM, Korevaar JC, Lambalk CB, van der VeenF.Effect of clomifene citrate plus metformin and clomifene citrate plus placebo on induction of ovulation in women with newly diagnosed polycystic ovary syndrome: randomised double blind clinical trial.BMJ. 2006; 332(7556):1485.

73. Creanga AA, Bradley HM, McCormick C, Witkop CT. Use of metformin in polycystic ovary syndrome: a meta-analysis. Obstet Gynecol. 2008; 111(4):959–968.

74. Hwu YM, Lin SY, Huang WY, Lin MH, Lee RK. Ultra-short metformin pretreatment for clomiphene citrate-resistant polycystic ovary syndrome. Int J Gynaecol Obstet. 2005; 90(1):39–43.

75. Isoda K, Young JL,et al. Metformin inhibits proinflammatory responses and nuclear factor-κ B in human vascular wall cells. Arterioscler. Thromb. vasc. Biol. 2006; 26:

76. Huang NL, Chiang SH, Hsueh CH, Liang YJ, Chen YJ & Lai LP. Metformin inhibits TNF-alpha-induced I kappa B kinase phosphorylation, I kappa B-alpha degradation and IL-6 production in endothelial cells through PI3K-dependent AMPK phosphorylation. International Journal of Cardiology. 2009; 134:

169–175.

77. ELMekkawi S.F., ELHosseiny A.S. Effect of Metformin Therapy on Serum Interleukin-6 and Interleukin-18 Levels in Patients with Polycystic Ovary Syndrome. Nature and Science. 2010; 8(9):23-26.

78. Azziz R, Ehrmann D, Legro RS, et al. Troglitazone improves ovulation and hirsutism in the polycystic ovary syndrome: a multicenter, double blind, placebo-controlled trial. J ClinEndocrinolMetab. 2001; 86(4): 1626–1632.

79. Tang T, Lord JM, Norman RJ, Yasmin E, Balen AH. Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, and d-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. Cochrane Database Syst Rev. 2010 ;(1):CD003053. Doi: 10.1002/14651858.CD003053.

80. Gysler M, March CM, Mishell DR, Bailey EJ. A decade's experience with an individualized clomifene treatment regimen including its effect on the postcoital test. Fertile Steril.1982;37:161-67.

81. MacGregorAH,Johnson JE, Bunde CA. Further clinical experience with clomifene citrate. Fertile Steril.1968;19:616-22

82. Imani B, Eijkemans MJ, teVelde ER. A nomogram to predict the probability of live birth after clomiphene citrate induction of

ovulation in normogonadotropicoligomenorrheic infertility. Fertile Steril. 2002; 77:91-97.

83. Van Santbrink EJ, Fauser BC. Ovulation induction in normogonadotropicanovulation (PCOS). Best. Pract. Res. Clin. Endocrinol. Metab. 2006;20:261-70.

84. Beck JI, Boothroyd C, et al. oral anti-oestrogens and medical adjuncts for subfertility associated with anovulation. Cochrane database Systematic Review.2005;1.

85. Homburg R, ArmarNA,et al. Influence of serumluteinising hormone concenterations on ovulation, conception and early pregnancy loss in polycystic ovary syndrome. Br Med J. 1998;297:1024-26.

86. KoustaE,White DM, et al. Modern use of clomiphene citrate in induction of ovulation. Human ReprodUptade. 1997;3:359-65.

87. Shoham Z, Borenstein R, et al. Hormonal profiles following clomiphene citrate therapy in conception and nonconception cycles. Clin Endocrinology. 1990;33:271-78.

88. Diamant YZ, Evron S. Induction of ovulation by combined clomiphene citrate and dexamethasone treatment in clomiphene citrate non-responders. EurJ ObstetGynaecol Biol.1981;11:335-40.

89. Daly DC, Walters CA, et al. A randomized study of dexamethasone in ovulation induction with clomiphene citrate. Fertile Streil. 1984; 41: 844-48.

90. Misso ML, Teede HJ, et al. Status of clomiphene citrate and metformin for infertility in PCOS. Trends EndocrinolMetab. 2012; 23(10):533-43.

91. Dasari P, Pranahita GK. The efficacy of metformin and clomiphene citrate combination compared with clomiphene citrate alone for ovulation induction in infertile patients with PCOS. J Hum Reprod Sci. 2009; 2(1): 18–22.

92. Levin E.R., Hammes S.R., Estrogens and Progestins. In :Brunton L.L., Chabner B.A., Knollman B.C.,. Goodman & Gilman's The Pharmacological Basis of Therapeutics. Twelth edition. China: The McGraw-Hill Companies,Inc; 2011.

93. Polycystic Ovary Syndrome: A guide to clinical management. Menstrual disturbances. USA: Taylor & Francis; 2005.

94. Falsetti L., Pasinetti E. Effects of long-term administration of an oral contraceptive containing ethinylestradiol and cyproteroneacetate on lipid metabolism in women with polycystic ovary syndrome.actaobstetrica et gynaecologicascandinavica. 1995; 74(1): 56-60.

95. SahaL,KaurS,et al. Pharmacotherapy of polycystic ovary

syndrome- an update. Fundamental and clinical Pharmacology.2011; 26(2012):54-62

96. Parker K.L., Schimmer B.P. Introduction to Endocrinology: The Hypothalamic-Pituitary Axis. In: Brunton L.L., Chabner B.A., Knollman B.C. Goodman & Gilman's The Pharmacological Basis of Therapeutics. Twelth edition. China: The McGraw-Hill Companies,Inc; 2011.

97. Buggs C, Rosenfield R.L.Polycystic Ovary Syndrome in Adolescence.EndocrinolMetabClin North Am. 2005 September ; 34(3).

98. Delbaere A, Smits G, Olatunbosun O, Pierson R, VassartG, Costagliola S. New insights into the pathophysiology of ovarian hyperstimulation syndrome. What makes the difference between spontaneous and iatrogenic syndrome? *Hum Reprod*. 2004; 19(3):486–489.

99. Christin-Maitre S, Hugues JN; Recombinant FSH Study Group. A comparative randomized multicentric study comparing the step-up versus step-down protocol in polycystic ovary syndrome. *Hum Reprod*. 2003; 18(8):1626–1631.

100. Filicori M, Cognigni GE, Taraborrelli S, et al. Luteinizing hormone activity in menotropins optimizes folliculogenesis and

treatment in controlled ovarian stimulation. *J Clin EndocrinolMetab*. 2001; 86(1):337–343.

101. Maggs DG, Buchanan TA, Burant CF, et al. Metabolic effects of troglitazone monotherapy in type 2 diabetes mellitus. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med*. 1998; 128(3):176–185.

102. Stadtmauer L.A, SarhanA,et al. The impact of a gonadotropin-releasing hormone antagonist on gonadotropin ovulation induction cycles in women with polycystic ovary syndrome: a prospective randomized study. Fertility and Sterility.2011; 95(1).

103. Kompanje EJ, Jansen TC, Van der hoven B, Bakker J.The first demonstration of lactic acid in human blood in shock by Johann Joseph Scherer (1814-1869)in January 1843. Intensive Care Med 2007; 33: 1967-1971.

104. The cell biology of inositol lipids and phosphates.Proceedings of the 2006 Biochemical Society Annual Symposium. Birmingham, United Kingdom. March 29-30, 2006. Biochemical Society symposium.2007(74): pp. 1-271.

105. Agranoff BW. Turtles All the Way: Reflections on myo-Inositol. J BiolChem 2009; 284: 21121-21126.

106. Anderson RJ. A Contribution to the chemistry of phytin: i. Composition of barium phytate and phytic acid. ii. A study of the properties of phytic acid and its decomposition products. Eighth paper on phytin. J BiolChem 1914; 17: 171-190.

107. Johnson LF, Tate ME. The structure of myo-inositol pentaphosphates. Ann N Y AcadSci 1969; 165: 526-532.

108. Clements RS, Darnell B. Myo-inositol content of common foods: development of a high-myoinositol diet. Am J ClinNutr 1980; 33: 1954-1967.

109. Ajumder AL, Biswas BB. Biology of inositols and phoshoinositides. Subcellular Biochemistry.Springer, 2006; p. 39.

110. Eisenberg F, Bolden AH, Loewus FA. Inositol formation by cyclization of glucose chain in rat testis. BiochemBiophys Res Commun 1964; 14: 419-424.

111. Eisenberg F, JR., Bolden AH. Reproductive tract as site of synthesis and secretion of inositol in the male rat. Nature 1964; 202: 599-600.

112. Voglmayr JK, Amann RP. The distribution of free myo-inositol in fluids, spermatozoa, and tissues of the bull genital tract and observations on its up-take by the rabbit epididymis. BiolReprod 1973;8: 504-513.

113. Lewin LM, Beer R. Prostatic secretion as the source of myo-inositol in human seminal fluid. FertilSteril 1973; 24: 666-670.

114. Ghafoorunissa. Effect of dietary protein & inositol on sperm metabolism & fructose content of male accessory sex organs of rat. Indian J ExpBiol 1976; 14: 564-566.

115. Spector R, Lorenzo AV. The origin of myo-inositol in brain, cerebrospinal fluid and choroid plexus. J Neurochem 1975; 25: 353-354.

116. Spector R, Lorenzo AV. Myo-inositol transport in the central nervous system. Am J Physiol 1975; 228: 1510-1518.

117. Bromberg WJ, Edlich RF. Joseph Larner's personal odyssey: search for the cause and cure of non-insulin-dependent diabetes mellitus. J Emerg Med 1994; 12: 681-684.

118. Larner J, Huang LC, et al. Insulin mediators: structure and formation. Cold Spring HarbSymp Quant Biol 1988; 53 Pt 2: 965-971.

119. Kennington AS, Hill CR, et al. Low urinary chiro-inositol excretion in non-insulin dependent diabetes mellitus. N Engl J Med 1990; 323: 373-378.

120. Loewus MW, Wright RW, et al. Activity of myo-inositol-1-phosphate synthase in the epididymal spermatozoa of rams. J ReprodFertil 1983; 69: 215-220.

121. O Ortmeyer HK, Bodkin NL, Lilley K, et al. Chiroinositol deficiency and insulin resistance. I. Urinary excretion rate of chiroinositol is directly associated with insulin resistance in spontaneously diabetic rhesus monkeys. Endocrinology 1993; 132: 640-645.

122. Pak Y, Huang LC, Lilley KJ, Larner J. In vivo conversion of [3H]myoinositol to [3H]chiroinositol in rat tissues. J BiolChem 1992; 267: 16904-16910.

123. Pak Y, Hong Y, et al. In vivo chiro-inositol metabolism in the rat: a defect in chiro-inositol synthesis from myo-inositol and an increased incorporation of chiro-[3H] inositol into phospholipid in the Goto-Kakizaki (G.K) rat. Mol Cells 1998; 8: 301-309.

124. Cohen P. The twentieth century struggle to decipher insulin signalling. Nat Rev Mol Cell Biol 2006; 7: 867-873.

125. Baillargeon JP, Nestler JE, et al. Greek hyperinsulinemic women, with or without polycystic ovary syndrome, display altered inositols metabolism. Hum Reprod 2008; 23: 1439-1446.

126. Baillargeon JP, Diamanti-kandarakis E, et al. Altered D-chiro-inositol urinary clearance in women with polycystic ovary syndrome. Diabetes Care 2006; 29: 300-305.

127. Nestler JE, Jakubowicz DJ, et al. Ovulatory and metabolic effects of D-chiroinositol in the polycystic ovary syndrome. N Engl J Med 1999; 340: 1314-1320.

128. Iuorno MJ, Jakubowicz DJ, et al. Effects of d-chiroinositol in lean women with the polycystic ovary syndrome. EndocrPract 2002; 8: 417-423.

129. Chiu TT, Tam PP. A correlation of the outcome of clinical in vitro fertilization with the inositol content and embryotrophic properties of human serum. J Assist Reprod Genet 1992; 9: 524-530.

130. Chiu TT, Rogers MS, Law EL, etal.Follicular fluid and serum concentrations of myo-inositol in patients undergoing IVF: relationship with oocyte quality. Hum Reprod 2002; 17: 1591-1596.

131. Papaleo E, Unfer V, et al. Myo-inositol in patients with polycystic ovary syndrome: a novel method for ovulation induction.GynecolEndocrinol 2007; 23: 700-703.

132. Gerli S, Papaleo E, et al. Randomized,double blind placebo-controlled trial: effects of myo-inositol on ovarian function and metabolic factors in women with PCOS. Eur Rev Med PharmacolSci 2007; 11: 347-354.

133. Genazzani AD, Lanzoni C, et al. Myo-inositol administration positively affectshyperinsulinemia and hormonal parameters in overweight patients with polycystic ovary syndrome. GynecolEndocrinol 2008; 24: 139-144.

134. Costantino D, Minozzi G, Minozzi E, GuaraldiC.Metabolic and hormonal effects of myo-inositol in women with polycystic ovary syndrome: a doubleblind trial. Eur Rev Med PharmacolSci 2009; 13:105-110.

135. Unfer V, Carlomagno G, Rizzo P, et al. Myo-inositol rather than D-chiro-inositol is able to improve oocyte quality in intracytoplasmic sperm injection cycles. A prospective, controlled, randomized trial. Eur Rev Med PharmacolSci 2011; 15: 452-457.

136. Chattopadhayay R, Ganesh A, et al. Effect of follicular fluid oxidative stress on meiotic spindle formation in infertile women with polycystic ovarian syndrome. GynecolObstet Invest 2010; 69: 197-202.

137. Carlomagno G, Unfer V, Roseff S. The D-chiroinositol paradox in the ovary. FertilSteril 2011; 95: 2515-2516.

138. Papaleo E, Unfer V, et al. Myo-inositol may improve oocyte quality in intracytoplasmic sperm injection cycles. A prospective, controlled, randomized trial. FertilSteril 2009; 91: 1750-1754.

139. Harwood K, Vuguin P, Dimartino-nardi J. Current approaches to the diagnosis and treatment of polycystic ovarian syndrome in youth. Horm Res 2007; 68: 209-217.

140. Isabella R, Raffone E. Does ovary need D-chiroinositol?J Ovarian Res 2012; 5: 14.

141. Galletta M, Grasso S, Vaiarelli A, Roseff SJ. Byebyechiro-inositol–myo-inositol: true progress in the treatment of polycystic ovary syndrome and ovulation induction. Eur Rev Med PharmacolSci 2011; 15: 1212-1214.

142. Minozzi M.,Nordio M., Pajalich R. The combined therapy myo-inositol plus D-Chiro-inositol, in a physiological ratio, reduces the cardiovascular risk by improving the lipid profile in PCOS patients. European Review for Medical and Pharmacological Sciences. 2013;17:537-540.

143. Fulghesu A.M, Ciampell M.,MuzjG,et al. N-acetylcysteine treatment improves insulin sensitivity in women with polycystic ovary syndrome.Fertil.Steril.2002;77:1128-35.

144. Kilic-Okman T.,Kucuk M. N-acetyl cysteine treatment for polycystic ovary syndrome. Int.J.Gynaecol.Obstet.2004; 85:296-97.

145. Rizk A.Y.,Bedaiway M.A., Al-Inany H.G. N-acetylcysteine is a novel adjuvant to clomiphene citrate in clomiphene citrate-

resistant patients with polycystic ovary syndrome. Fertil.Steril.2005; 83:367-70.

146. Salehpour S., Tohidi M., etal.N-acetylcysteine, a novel remedy for polycystic ovarian syndrome. Int.J.Fertil. Steril.2009;3:66-73.

147. Gonzalez F.,RoteN.S.,et al. Reactive oxygen species induced oxidative stress in the development of insulin resistance and hyperandrogenism in polycystic ovary syndrome. J. Clin. Endocrinol. Metab. 2006;91:336-40.

148. Yildirim B., DemirS.,et al. Lipid peroxidation in follicular fluid of women with polycystic ovary syndrome during assisted reproduction cycles. J. Reprod. Med.2007;52:722-26

149. Sun C.L., QiaoJ.,et al. Expression of novel apoptosis-related protein PDCD5 in granulosa cells of polycystic ovary syndrome. Beijing Da.Xue.Xue. Bao.2005;37:476-93.

150. Tamura H.,NakamuraY.,et al. Melatonin and the ovary:physiological and pathophysiological implications. Fertil.Steril.2009;92:328-43.

151. Kodaman P.H.,DulebaA.J.Statins:do they have potential in the treatment of polycystic ovary syndrome? Semin. Reprod. Med. 2008;26:127-38.

152. Balen AH.PCOS- Medical or Surgical treatment? In: Templeton A, Cooke I, O'Brien PMS eds. Evidence-based fertility treatment,RCOG study Group.London:RCOG Press,1998:157-77.

153. Al-Fadhli R, Tulandi T. Laparoscopic treatment of polycystic ovaries:is its place diminishing? *CurrOpinObstet Gynecol*. 2004;16(4):295–298.

154. Wild S, Pierpoint T, et al. Long term consequences of polycystic ovary syndrome: results of a 31 year follow-up study. Human Fertility. 2000; 3(2): 101-105.

155. Barber T.M., McCarthy M.I., et al. Obesity and plycystic ovary syndrome. Clinical Endocrinology. 2006; 65(2): 137-145.

156. Badawy A, Elnashar A. Treatment options for Polycystic Ovary Syndrome. International Journal of Women's Health.2011.

157. Samy N, Hashim M, et al. Clinical significance of inflammatory markers in polycystic ovary syndrome: Their relationship to insulin resistance and Body Mass Index. Disease Markers. 2009; 26:163-170.

158. Hector F., Escobar-Morreale, et al. Serum Interleukin-18 Concentrations are increased in the polycystic ovary syndrome: Relationship to insulin resistance and to obesity. The Journal of

Clinical Endocrinology & metabolism. 2004; 89: 806-811.

159. Escobar-Morreale H.F., Villuendas G, et al. Obesity, and not insulin resistance, is the major determinant of serum inflammatory cardiovascular risk marker in premenopausal women. Diabetologia. 2003; 46: 625-633.

ABBREVIATIONS

Ab	–	Antibody
AMPK	–	Adenosine Monophosphate Kinase
AGE	–	Advanced Glycated End products
ASRM	–	American Society of Reproductive Medicine
CC	–	Clomiphene Citrate
CRP	–	C - reactive protein
FSH	–	Follicle Stimulating Hormone
GnRH	–	Gonadotropin Releasing Hormone
GnRHa	–	Gonadotropin Releasing Hormone agonist
hs-CRP	–	High sensitivity C- reactive protein
HRP	–	Horse Radish peroxidase
IL-6	–	Interleukin-6
IL-18	–	Interleukin-18
IL-8	–	Interleukin-8
IL-1β	–	Interleukin-1β
IR	–	Insulin Resistance
IVF	–	In Vitro Fertilization
LH	–	Luteinizing Hormone
NAC	–	N-Acetyl Cysteine
NFκB	–	Nuclear Factor kappa B

PCOS	–	Polycystic Ovary Syndrome
ROS	–	Reactive Oxygen Species
Std.	–	Standard

CASE REPORT FORM

NAME :

AGE :

O.P. NUMBER :

HEIGHT :

WEIGHT:

HISTORY:

DRUG HISTORY:

USG FINDINGS:

INTERLEUKIN-6:

INTERLEUKIN-18:

hs CRP:

FSH :LH (if done) :

PSG Institute of Medical Science and Research, Coimbatore
Institutional Human Ethics Committee
INFORMED CONSENT FORMAT FOR RESEARCH PROJECTS

(strike off items that are not applicable)

I,Senthurselvi R. am carrying out a study on the topic: A CROSS SECTIONAL STUDY ON THE STATUS OF INFLAMMATORY MARKERS IN POLYCYSTIC OVARY SYNDROME(PCOS) PATIENTS IN INDIAN POPULATION as part of my research project being carried out under the aegis of the Department of PHARMACOLOGY.

My research guide is: Dr.K.BHUVANESWARI,Professor and Head of the department,Pharmacology.

The justification for this study is: PCOS is prevalent nowadays. There are no studies demonstrating the role of inflammation in patients with PCOS in Indian population and also how the drugs prescribed for PCOS influences the inflammation.

The objectives of this study are:

Primary Objective: To assess the status of inflammation in patients with PCOS

Secondary Objective: to assess the influence of drugs prescribed for PCOS on inflammation

Sample size:90..

Study volunteers / participants are (specify population group & age group): Patients diagnosed to have PCOS and also healthy volunteers with age group between18-45 years.

Location:the study will be conducted in PSGIMS&R hospital.

We request you to kindly cooperate with us in this study. We propose collect background information and other relevant details related to this study. We will be carrying out:

Initial interview (specify approximate duration):20 minutes.

Data collected will be stored for a period of five years. We will not use the data as part of another study.

Health education sessions: Number of sessions:one. Approximate **duration** of each session:

20 minutes.

Clinical examination (Specify details and purpose): Height and Weight for BMI

Blood sample collection: Specify quantity of blood being drawn: 5 ml.

No. of times it will be collected: 1

Whether blood sample collection is part of routine procedure or for research (study) purpose:

Research purpose

Specify **purpose**, discomfort likely to be felt and side effects, if any: pain while pricking

Whether blood sample collected will be stored after study period: No, it will be destroyed

Whether blood sample collected will be sold: No

Whether blood sample collected will be shared with persons from another institution: No

Medication given, if any, duration, side effects, purpose, benefits: No

Benefits from this study: PCOS is prevalent nowadays. There are no studies demonstrating the role of inflammation in patients with PCOS in Indian population and also how the drugs prescribed for PCOS influences the inflammation.

Risks involved by participating in this study: No risks

How the **results** will be used: Clinical meeting at PSG Hospitals
Dr.MGR Medical University dissertation

If you are uncomfortable in answering any of our questions during the course of the interview / biological sample collection, **you have the right to withdraw from the interview / study at anytime.** You have the freedom to withdraw from the study at any point of time. Kindly be assured that your refusal to participate or withdrawal at any stage, if you so decide, will not result in any form of compromise or discrimination in the services offered nor would it attract any penalty. You will continue to have access to the regular services offered to a patient. You will **NOT** be paid any remuneration for the time you spend with us for this interview / study. The information provided by you will be kept in strict confidence. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only.You will be informed about any significant new findings - including adverse events, if any, – whether directly related to you or to other participants of this study, developed during the course of this research which may relate to your willingness to continue participation.

Consent: The above information regarding the study, has been read by me/ read to me, and has been explained to me by the investigator/s. Having understood the same, I hereby give my consent to them to interview me. I am affixing my signature / left thumb impression to indicate my consent and willingness to participate in this study (i.e., willingly abide by the project requirements).

Signature / Left thumb impression of the Study Volunteer / Legal Representative:

Signature of the Interviewer with date: Witness:

Contact number of PI:

Contact number of Ethics Committee Office: 0422 2570170 Extn.: 5818

ஒப்புதல் படிவம்

தேதி:

இரா. செந்தூர் செல்வி ஆகிய நான், **PSG** மருத்துவக் கல்லூரியின் மருந்தியல் துறையின் கீழ் மேற்படிப்பு படித்துக்கொண்டு இருக்கின்றேன். நான், இந்தியர்களின் பல கருமுட்டை பை இணைப்போக்கில் அழற்ச்சி தன்மையை அறிந்துக்கொள்ளுதல் என்ற தலைப்பில் ஆய்வு மேற்கொண்டுள்ளேன்.

என் ஆய்வு வழிகாட்டி: K. புவனேஸ்வரி, தலைமை மருந்தியல் துறை

ஆய்வு மேற்கொள்வதற்கான அடிப்படை : பல கருமுட்டை பை இணைப்போக்கு என்ற நோய் பரவலாக உள்ளது. இந்தியர்களின் அழற்சி தன்மையை அறிந்து கொள்ளும் ஆராய்ச்சி மற்றும் அதற்கு தரும் மருந்துகள் எவ்வாறு அழற்சியை நீக்குகிறது என்ற ஆராய்ச்சியும் மிகக் குறைவு. எனவே, இந்தியர்களின் அழற்சியின் தன்மையை இந்நோயில் கண்டறிவதே இந்த ஆய்வின் நோக்கம்.

ஆய்வின் நோக்கம்: இந்தியர்களின் அழற்சியின் தன்மையை இந்நோயில் கண்டறிவதே இந்த ஆய்வின் நோக்கம்.

ஆய்வில் பங்குபெறும் நபர்களின் எண்ணிக்கை: 90

ஆய்வு மேற்கொள்ளும் இடம்: PSG மருத்துவமனை

ஆய்வின் பலன்கள்: பல கருமுட்டை பை இணைப்போக்கு என்ற நோய் பரவலாக உள்ளது. இந்தியர்களின் அழற்சி தன்மையை அறிந்து கொள்ளும் ஆராய்ச்சி மற்றும் அதற்கு தரும் மருந்துகள் எவ்வாறு அழற்சியை நீக்குகிறது என்ற ஆராய்ச்சியும் மிகக் குறைவு. எனவே, இந்தியர்களின் அழற்சியின் தன்மையை இந்நோயில் கண்டறிவதே இந்த ஆய்வின் நோக்கம்.

-- 2 –

ஆய்வினால் ஏற்படும் அசௌகரியங்கள் / பக்க விளைவுகள்: எதுவுமில்லை.

இந்த ஆய்வில் கிடைக்கும் தகவல்கள் **5** வருடங்கள் பாதுகாக்கப்படும். இவை வேறு எந்த ஆய்விற்க்கும் பயன்படுத்தப்பட மாட்டாது. எந்த நிலையிலும் உங்களைப் பற்றிய தகவல்கள் யாருக்கும் தெரிவிக்கப்பட மாட்டாது. அவை இரகசியமாக வைக்கப்படும்.
இந்த ஆய்வில் பங்கேற்க ஒப்புக்கொள்ளுவதால் எந்த விதமான பலனும் உங்களுக்குக் கிடைக்காது. எந்த நேரத்தில் வேண்டுமானாலும் ஆய்விலிருந்து விலகிக்கொள்ளும் உரிமை உங்களுக்கு உண்டு. ஆய்விலிருந்து விலகிக்கொள்வதால் உங்களுக்கு அளிக்கப்படும் சிகிச்சையில் எந்தவித மாற்றமும் இருக்காது.
இந்த ஆராய்ச்சிக்காக உங்களிடம் சில கேள்விகள் கேட்கப்படும் / சில இரத்த மாதிரிகள் அல்லது திசு மாதிரிகள் எடுக்கப்படும்.

ஆய்வாளரின் கையொப்பம் :

தேதி :

ஆய்வுக்குட்படுபவரின் ஒப்புதல்:

நான் இந்த ஆராய்ச்சியின் நோக்கம் மற்றும் அதன் பயன்பாட்டினைப் பற்றி தெளிவாகவும், விளக்கமாகவும் தெரியப்படுத்தப்பட்டுள்ளேன். இந்த ஆராய்ச்சியில் பங்கு கொள்ளவும், இந்த ஆராய்ச்சியின் மருத்துவ ரீதியான குறிப்புகளை வரும் காலத்தில் உபயோகப்படுத்திக் கொள்ளவும் முழு மனதுடன் சம்மதிக்கிறேன்.

ஆய்வுக்குட்படுபவரின் பெயர், முகவரி :

கையொப்பம் :

தேதி :

ஆய்வாளரின் தொலைபேசி எண் :

மனித நெறிமுறைக் குழு அலுவலகத்தின் தொலைபேசி எண்: 0422 2570170 **Extn** : 5818

PSG Institute of Medical Science and Research, Coimbatore
Institutional Human Ethics Committee
INFORMED CONSENT FORMAT FOR RESEARCH PROJECTS

(strike off items that are not applicable)

I,Senthurselvi R. am carrying out a study on the topic: A CROSS SECTIONAL STUDY ON THE STATUS OF INFLAMMATORY MARKERS IN POLYCYSTIC OVARY SYNDROME(PCOS) PATIENTS IN INDIAN POPULATION as part of my research project being carried out under the aegis of the Department of PHARMACOLOGY.

My research guide is: Dr.K.BHUVANESWARI,Professor and Head of the department,Pharmacology.

The justification for this study is: PCOS is prevalent nowadays. There are no studies demonstrating the role of inflammation in patients with PCOS in Indian population and also how the drugs prescribed for PCOS influences the inflammation.

The objectives of this study are:

Primary Objective: To assess the status of inflammation in patients with PCOS

Secondary Objective: to assess the influence of drugs prescribed for PCOS on inflammation

Sample size:90..

Study volunteers / participants are (specify population group & age group): Patients diagnosed to have PCOS and also healthy volunteers with age group between18-45 years.

Location:the study will be conducted in PSGIMS&R hospital.

We request you to kindly cooperate with us in this study. We propose collect background information and other relevant details related to this study. We will be carrying out:

Initial interview (specify approximate duration):20 minutes.

Data collected will be stored for a period of five years. We will not use the data as part of another study.

Health education sessions: Number of sessions:one. Approximate **duration** of each session:

20 minutes.

Clinical examination (Specify details and purpose): Height and Weight for BMI

Blood sample collection: Specify quantity of blood being drawn: 5 ml.

No. of times it will be collected: 1

Whether blood sample collection is part of routine procedure or for research (study) purpose:

Research purpose

Specify **purpose**, discomfort likely to be felt and side effects, if any: pain while pricking

Whether blood sample collected will be stored after study period: No, it will be destroyed

Whether blood sample collected will be sold: No

Whether blood sample collected will be shared with persons from another institution: No

Medication given, if any, duration, side effects, purpose, benefits: No

Benefits from this study: PCOS is prevalent nowadays. There are no studies demonstrating the role of inflammation in patients with PCOS in Indian population and also how the drugs prescribed for PCOS influences the inflammation.

Risks involved by participating in this study: No risks

How the **results** will be used: Clinical meeting at PSG Hospitals
Dr.MGR Medical University dissertation

If you are uncomfortable in answering any of our questions during the course of the interview / biological sample collection, **you have the right to withdraw from the interview / study at anytime.** You have the freedom to withdraw from the study at any point of time. Kindly be assured that your refusal to participate or withdrawal at any stage, if you so decide, will not result in any form of compromise or discrimination in the services offered nor would it attract any penalty. You will continue to have access to the regular services offered to a patient. You will **NOT** be paid any remuneration for the time you spend with us for this interview / study. The information provided by you will be kept in strict confidence. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only. ***You will be participating in this study as a healthy volunteer to assess the normal limits***. You will be informed about any significant new findings - including adverse events, if any, – whether directly related to you or to other participants of this study, developed during the course of this research which may relate to your willingness to continue participation.

Consent: The above information regarding the study, has been read by me/ read to me, and has been explained to me by the investigator/s. Having understood the same, I hereby give my consent to them to interview me. I am affixing my signature / left thumb impression to indicate my consent and willingness to participate in this study (i.e., willingly abide by the project requirements).

Signature / Left thumb impression of the Study Volunteer / Legal Representative:

Signature of the Interviewer with date: Witness:

Contact number of PI:
Contact number of Ethics Committee Office: 0422 2570170 Extn.: 5818